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Deep-Sequencing Analysis of DF-1 Cell Transcriptome Response to Infection with Newcastle Disease Virus of Duck

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Abstract: Newcastle Disease (ND) is caused by Newcastle Disease Virus (NDV) with a respiratory and digestive mucosal bleeding for typical symptoms. It is an acute highly contacting disease. NDV could multiplicate in DF-1 cells. NDV from waterfowl is different from former NDV from chicken, it lead waterfowl dead. But the molecular mechanisms of waterfowl NDV infected still are poorly understood. In this study researchers employed the illumina genome analyzer platform to perform genome-wide Digital Gene Expression (DGE), a tagbased high-throughput transcriptome sequencing method analysis of DF-1 cells infected NDV-YC. Researchers could reach a sequencing depth of 5.0-6.1 million tags per library and found >8000 genes to be differentially expressed during NDV-YC infection processes. NDV-YC notably identified genes involved in I-kappaB kinase/NF-kappaB and TGF-beta signaling pathway. Further analysis employed Gene Ontology (GO) analysis cell part, binding and biological regulation terms are main items, respectively. The data benefit for better understanding the molecular mechanism of NDV-YC infected and provide a basis for further *in vivo* study and clinical trials.

Key words: Digital Gene Expression (DGE), NDV-YC, pathway, GO analysis, symptoms

INTRODUCTION

Newcastle Disease Virus (NDV) which causes a highly infectious and widespread disease in poultry (Miller *et al*., 2010; Alexander and Allan, 1974) is regarded in China as a notifiable disease by the Office International Des Epizooties (OIE) and results in severe economic losses (Cai *et al*., 2011; OIE, 2009). NDV is a member of the Avian Paramyxovirus serotype 1 (APMV-1), belonging to the genus *Avulavirus* within the family Paramyxoviridae. The NDV genome consists of a single stranded negative-sense RNA with 15186, 15192 or 15198 Nucleotides (nt) that encodes six structural proteins Haemagglutinin-Neuraminidase (HN), Fusion (F), Matrix (M) Proteins, Nucleoprotein (NP), Phosphoprotein (P) and RNA polymerase (L) (Collins *et al*., 1989; Chu *et al*., 1982; Hussein *et al*., 2005). Paramyxovirus infection is initiated by the action of two envelope glycoproteins, one of which Haemagglutinin-Neuraminidase (HN), mediates attachment to the host cell receptor. The other glycoprotein the Fusion (F) protein is responsible for virus penetration into the host cell and virus-induced cell fusion and haemolysis. HN proteins generally contain six potential glycosylation sites (119, 341, 433, 481, 508 and 538), of which five are conserved in paramyxovirus. NDV has been found and isolated since 1926, it has widespreaded throughout the world and the host range has expanded. Water fowl become the infection host of ND from recently in China. NDV from waterfowl which is nature infected and caused waterfowl morbidity death is different from former NDV from chicken. From 1997, in Guangdong and Jiangsu, there were many gooses infected NDV one after another (Cai *et al*., 2011). The disease caused serious morbidity, death and it gradually spread to the East China. Separated virus from the morbidity goose groups reserchers conformed that virus was agreed with biology characteristic and the nucleic acid sequence of NDV. It was very easy to duplicate sickness artificially.

Next-generation high-throughput deep-sequencing technology such as Digital Gene Expression tag profiling (DGE) has recently been adapted for transcriptome analysis (Xiao *et al*., 2010; Hegedus *et al*., 2009). This direct sequencing methodology allows for the identification of millions of short RNAs in a sample and of Differentially Expressed Genes (DEGs) without the need for prior annotations (Wang *et al*., 2009; Cloonan and Grimmond, 2008; Morozova and Marra, 2008).

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Sequencing-based methods generate absolute gene expression measurements and avoid many of the inherent limitations of earlier microarray-based assays (Wilhelm *et al*., 2008).

In order to clarify molecular mechanism of pathogenicity researchers worked from host by illumina genome analyzer platform to perform a digital gene expression analysis of the DF-1 cells transcriptome response to NDV-YC from duck infection. Apoptosis played an important role in the oncolytic processs induced by NDV. Go and Pathway analysis showed that apoptosis signaling pathways induced by NDV. The research systematically investigate the oncolytic efficacy and apoptotic mechanisms induced by NDV strains providing a basis for further *in vivo* study and clinical trials.

MATERIALS AND METHODS

Virus cells: DF-1 cells were provided by the collection of cell lines in the College of Veterinary Medicine, South China Agricultural University, China. NDV-YC which was isolated from a duck farm in the Guangdong province of China was harvested in 9-10 days old Specific Pathogen Free (SPF) chicken embryos (Guangdong Dahuanong Animal Health Products Co., Ltd.) by using limiting dilution standard procedures and plaque-purified three times on primary chicken embryo fibroblasts. It was provided by the Animal Infectious Diseases Laboratory of South China Agricultural University.

Sample preparation: Cells that were cultured in a conventional source of amino acids were used as mock infected controls in parallel while DF-1 cells were infected with NDV at a Multiplicity of Infection (MOI) of 1. The DF-1 cells were harvested at 24 h post-infection (hpi). After washing three times with ice-cold Phosphate-Buffered Saline (PBS) the total RNA of the collected cells were obtained using trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the recommendations of the manufacturer. The RNA samples were treated with DNase I (TaKaRa, Japan) to clean up residual genomic DNA. RNA integrity and concentration were evaluated by Agilent 2100 Bioanalyzer (Agilent technologies).

Digital Gene Expression tag profiling (DGE): The main reagents and supplies are illumina gene expression sample prep kit and solexa sequencing chip (flowcell) and the main instruments are illumina cluster tation and Illumina HiSeqTM 2000 System.

Sequence tag preparation was done using the Digital Gene Expression Tag Profiling kit (Illumina) according to the manufacturer's protocol. A total of cDNA per tag library was purified and sequenced by the illumina genome analyzer II system (ServiceXS, Leiden, the Netherlands). Image analysis, base calling and tag counting were performed using the Solexa Automated Pipelin. For analysis, adaptor tags were filtered and low-quality tags and tags with a copy number of one were excluded. The clean tags were classified according to their copy-number in the library. The percentage of the total clean tags and analyzed saturation of the library were showed. Researchers generated the clean tags and mapped to the reference sequences in the chicken genome and virus reference sequences (ftp://ftp.ncbi.nih.gov/genomes/Gallus gallus/RNA/rna.fa.gz, ftp://ftp.ncbi.nih.gov/genomes/Gallus gallus/Assembled chromosomes, all found in the GenBank database) that only 1bp mismatch accepted. Tags that only one matched to unambiguous tags with a criterion were analyzed (Morrissy *et al*., 2009; Hoen *et al*., 2008).

Differentially Expressed Genes (DEGs) detection: To compare DEGs across samples (YC/C), the number of raw clean tags in each library was normalized to the number of TPM (Transcripts Per Million clean tags) to obtain normalized gene expression levels. The detection of DEGs or tags across samples was performed as previously described (Audic and Claverie, 1997) researchers should preset the FDR to a number no larger than 0.01 (Benjamini and Yekutieli, 2001). FDR$\leq$0.001 and an absolute value of log2 ratio$\geq$1 was set as the threshold to judge the significance of gene expression difference. More stringent criteria with smaller FDR and bigger fold-change value can be used to identify DEGs.

Cluster analysis of the DEG pattern: Genes with similar expression patterns usually mean functional correlation. Researchers perform cluster analysis of gene expression patterns with Cluster (Eisen *et al*., 1998) Software and Java Treeview (Saldanha, 2004) Software.

Gene Ontology (GO) functional enrichment analysis for DEGs: In gene expression profiling analysis GO enrichment analysis of functional significance applies hypergeometric test to map all differentially expressed genes to terms in GO database looking for significantly enriched GO terms in DEGs comparing to the genome background. The calculating equation is:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

Where:

N = The number of all genes with GO annotation
n = The number of DEGs in N
M = The number of all genes that are annotated to the certain GO terms
m = The number of DEGs in M

The GO functional enrichment analysis also integrates the clustering analysis of expression patterns. Thus, researchers can easily get the expression patterns of DEGs annotated to the given GO-term.

Pathway enrichment analysis for DEGs: Different genes usually cooperate with each other to exercise their biological functions. Pathway-based analysis helps to further understand genes biological functions. KEGG is the major public pathway-related database (Yi *et al*., 2006). Pathway enrichment analysis identifies significantly enriched metabolic pathways or signal transduction pathways in DEGs comparing with the whole genome background. The calculating formula is the same as that in GO analysis. Here, N is the number of all genes that with KEGG annotation, n is the number of DEGs in N, M is the number of all genes annotated to specific pathways and m is number of DEGs in M.

RESULTS AND DISCUSSION

Cytopathic effect of the NDV-YC-infected DF-1 cells: After YC infection, the affected DF-1 cells exhibited obvious CPE after 24 h inoculation. CPE was observed as the most variable morphological alterations of the cells including cell fusion, degeneration and rounding, granularity and vacuolization (Fig. 1a). Occasionally plaques of dead cells were present (Fig. 1b). Uninfected cells stained more densely than infected cells.

Analysis of DGE libraries: Global gene expression profiles were analyzed using the Solexa/Illumina DGE system a tag-based transcriptome sequencing method, in order to investigate the regulation of the host response to the NDV-YC virus. Researchers sequenced two DF-1 cells DGE libraries from uninfected C and NDV-YC -infected using massively parallel sequencing on the Illumina platform. The major characteristics of these two libraries were shown in Table 1.

Researchers obtained approximately 5.6 million total sequence tags per library with 291822 distinct tag sequences. After researchers filtered out adaptor tags, low quality tags and tags of copy number = 1, it produced 5.4 million total clean sequence tags with 111003 distinct clean tag sequences. The C library had the higher number

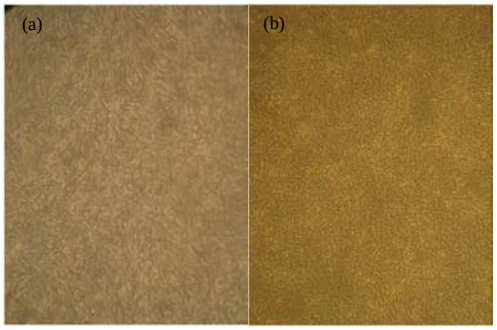

Fig. 1: Morphological CPE of DF-1 cells infected with the NDV-YC on 24 h post-infection. a) YC-NDV-infected DF-1 cells; b) Normal cells

Table 1: Major characteristics of DGE libraries and tag mapping to the UniGene transcript database. All mapping represents the number of all tags mapped to the UniGene virtual tag database, unambiguous mapping represents the number of unambiguous tags mapped to the UniGene virtual tag database, unambiguous tags indicate the tags matched only to one gene

	C		YC	
Data	Distinct tag	Total tag	Distinct tag	Total tag
Raw data	321990	6194034	261654	5000000
Tags containing N	19213	51720	8353	17443
Adaptors	95	101	0	0
Tag CopyNum <2	178787	178787	155190	155190
Clean tag	123895	5963426	98111	4827367
CopyNum >2	123895	5963426	98111	4827367
CopyNum >5	50704	5758612	41179	4667048
CopyNum >10	33956	5631747	27734	4565226
CopyNum >20	22487	5464369	18589	4431628
CopyNum >50	12652	5146769	10531	4172995
CopyNum >100	7741	4797796	6398	3879496
Tag mapping				
All mapping	51629	3341942	42584	2699478
Unambiguous mapping	47976	3012617	39722	2452611
Unknown tag	24597	1247955	16051	905688

of both total sequence tags and distinct sequence than the YC libraries. Analysis of capacity of libraries revealed that new emerging distinct tags were gradually reduced as the total sequence tags were increased (Fig. 2). The sequencing saturation analysis can be performed to check whether the number of detected genes keeps increasing when sequencing amount total tag number increases. When sequencing amount reaches 2 million or higher, the number of detected genes almost ceases to increase (Fig. 3).

Analysis of tag mapping: For tag mapping researchers preprocessed one reference tag database that included 19208 sequences from gallus unigene. To get the reference tags researchers used nlaIII to digest the samples and took all the CATG+17 tags as the gene's reference tags. Researchers obtained 154327 total

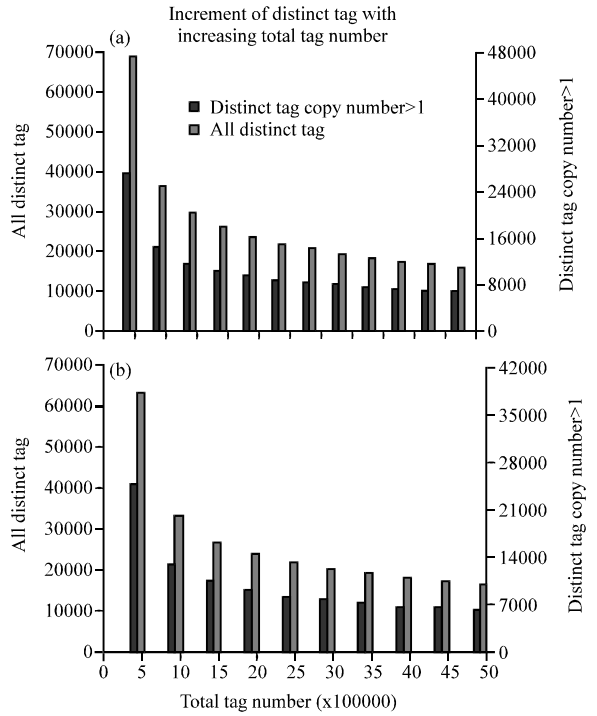

Fig. 2: Saturation of DGE libraries: Analysis of capacity of libraries showed that new emerging distinct tags were gradually reduced with increasing of total sequence tags when the number of sequencing tags was big enough. a) C; b) YC

Table 2: Summary of antisense transcripts

No of UniGene	C	YC
Sense	31213	25401
Antisense	16763	14321
Overlap	10559	8564
Antisense-specific	6204	5757
Sense/Antisense	1.862	1.7737

reference tag sequences including 146895 unambigous tag sequences. About polymorphism across samples, only one mismatch was allowed in each alignment. According to the criteria, 41.46-43.40% of distinct clean tags mapped to the unigene virtual tag database, 40.49-38.72% of the distinct clean tags mapped unambiguously to the Unigene and 16.36-19.85% of the distinct clean tags didn't map to the unigene virtual tag database (Table 1). Researchers can distinguish transcripts by solexa sequencing. It revealed that overlap transcription were 8564, 10559 of all detectable unigene and 5757, 6204 antisense-stand specific transcripts (Table 2). By comparison, the ratio of sense to antisense strand of the transcripts was approximately 1.8179 for all libraries. This suggests that in spite of the high number of antisense mapping events detected, the transcriptional regulation in the NDV pathogenicity acts most strongly on the sense strand.

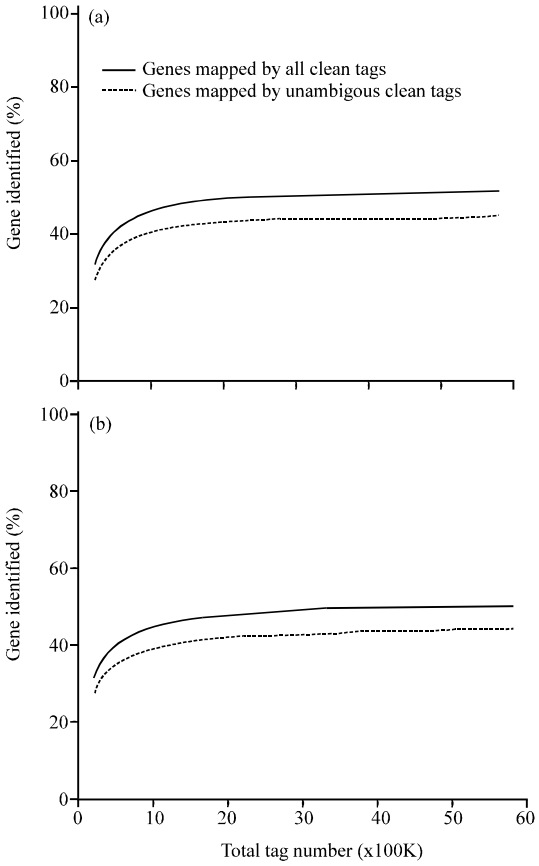

Fig. 3: Sequencing saturation analyses: The sequencing saturation analysis can be performed to check whether the number of detected genes keeps increasing when sequencing amount total tag number increases. a) C; b) YC

Identification of Differentially Expressed Genes (DGEs): To gain the global transcriptional changes in NDV-YC infected DF-1 cells researchers applied the method described previously (Audic and Claverie, 1997) to identify DEGs from the normalized DGE data by pairwise comparisons between two samples (C/YC). Results showed that 1922 genes had $p<0.005$, False Discovery Rate (FDR) <0.01 and estimated absolute log 2 change <1 in at least one of the pairwise comparisons (Fig. 4), C versus YC. Statistical analysis confirmed 1922 genes differentially transcribed in C versus YC which were declared to be differentially expressed during infection course (Table 1).

Gene Ontology (GO) and clusters analysis: GO assignments were used to classify the functions by the predicted 9800 genes. With the reference sequence alignment, >2000 genes can be categorized into

groups (Fig. 5). GO has three ontologies molecular function, cellular component and biological process. In the GO classification, cell part binding and biological regulation terms are main items, respectively. Researchers also noticed a large quantity of genes from categories of organelle catalytic activity and catabolic process and only a few genes from terms of nucleoplasm part and ligase activity and I-kappaB kinase/NF-kappaB cascade. GO functional enrichment analysis and the clustering analysis of expression patterns were integrated. Researchers can easily get the expression patterns of DEGs annotated to the given GO-term. In total, out of 1133 cluster, the cluster for I-kappaB kinase/NF-kappaB cascade represents the group 10 out of 1133 genes, 0.9% (Fig. 6).

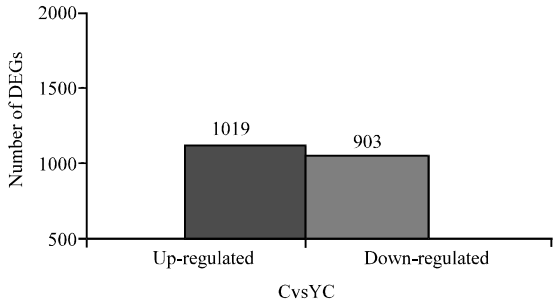

Fig. 4: Different expressed genes between libraries. All genes mapped to the genome were examined for expression differences between the different libraries. Numbers of differentially expressed genes represent across sense transcripts using threshold values rate (FDR) <0.01 and log 2 change <1 for controlling false discovery rates

Signaling pathway analysis: To characterize the functional consequences of gene expression changes associated with infection with NDV-YC, researchers performed pathway analysis of DE genes based on the KEGG (Kanehisa *et al*., 2004) database by two-side Fisher's exact test. Researchers chose only significant pathway categories that had a p-value of <0.05 and an FDR of <0.05. As shown in Fig. 7, the significant signaling pathways include regulation of actin cytoskeleton, pathways in cancer, MAPK signaling pathway, TGF-beta signaling pathway, toll-like receptor signaling pathway, p53 signaling pathway, Cell Adhesion Molecules (CAMs), etc.

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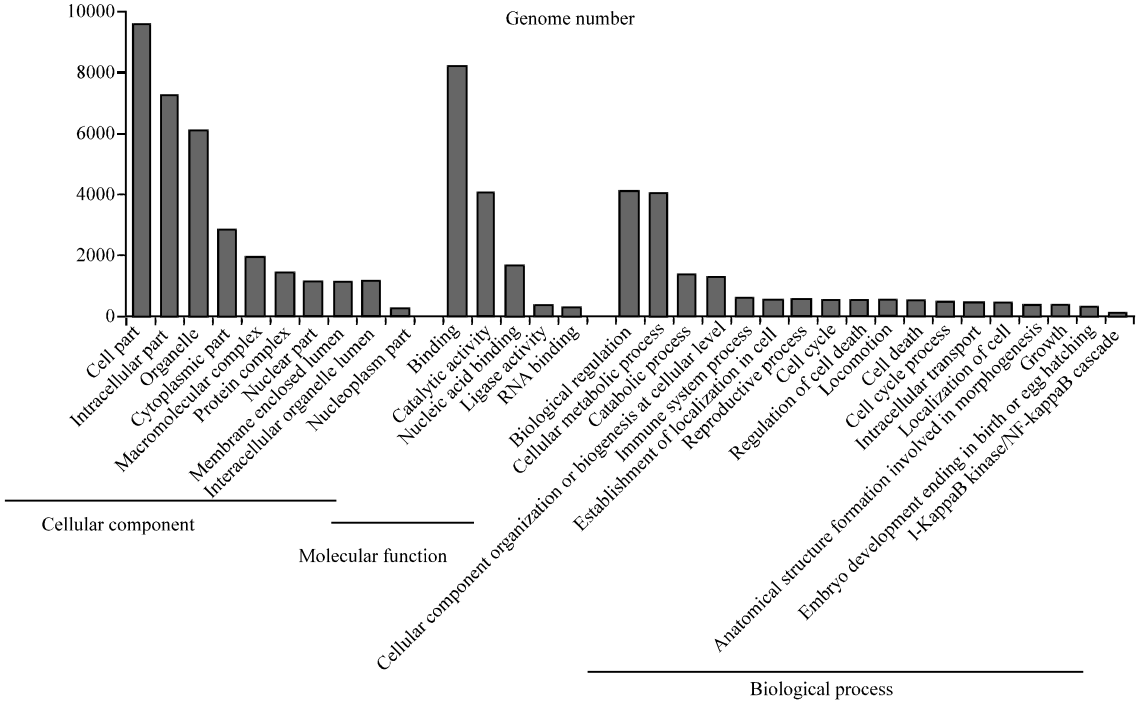

Fig. 5: Histogram presentation of gene ontology classification. The results are summarized in three main categories: biological process, cellular component and molecular function. The right y-axis indicates the number of genes in a category. The left y-axis indicates the percentage of a specific category of genes in that main category

variable morphological alterations of the DF-1 cells (Ravindra *et al*., 2009). Although great efforts have been made by many researchers, the molecular basis of NDV from waterfowl which is nature infected and caused duck morbidity death is largely unknown. Here, we report on the first genome-wide host cell transcriptional response to NDV-YC infection using Solexa/Illumina's Digital gene Expression (DGE) method which is a new tag-based high throughput transcriptome deep sequencing method. Given the nature of the methodology of Illumina's DGE system researchers make representative samples for deep sequencing analysis. Researchers could reach a sequencing depth of 5.0-6.1 million tags per library and found >8000 genes to be differentially expressed during NDV-YC infection processes. Although, others studies have admix biological replicates for library construction and deep sequencing (Glazov *et al*., 2008; Hegedus *et al*., 2009), resulting in the lack of biological replicate, it may cause fuzzy to the impact of variations in admix samples.

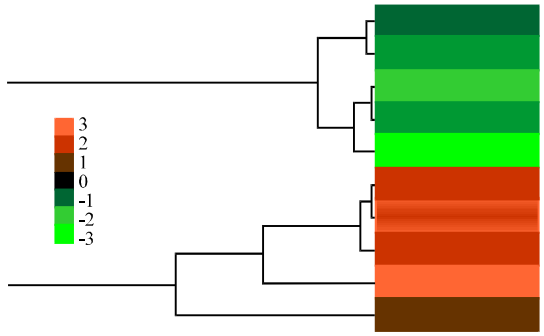

Fig. 6: The cluster of I-kappaB kinase/NF-kappaB cascade. It represents the group 10 out of 1133 genes

In the cell multiplication, the differentiation, senile and perish weakly and so on the vital activities, the transcriptome has the type and quantity difference. At the same time, the external environment stimulation including stimulations and so on biological or non-biotic factor can obviously affect the mRNA expressed to the protein. Although, protein is the physiological function performer and biological phenomena, GO and pathway analyses by DGE can even more draw close to grasps the life the phenomenon from mRNA and find the vital activity rule. The research through the establishment Solexa/Illumina's Digital Gene Expression (DGE) platform researchers appraised these difference tag to libraries and analyzes its function, to further verify the NDV pathogenesis mechanism to provide the experiment to rest on. The research discovered that NDV-YC infected DF-1 cell had the many changes in including the cell skeleton, the signal conduction, the macro-molecule synthesis and the biological metabolism, etc.

The cell skeleton has three dimensional network structure which are the micro silk (microfilaments), the

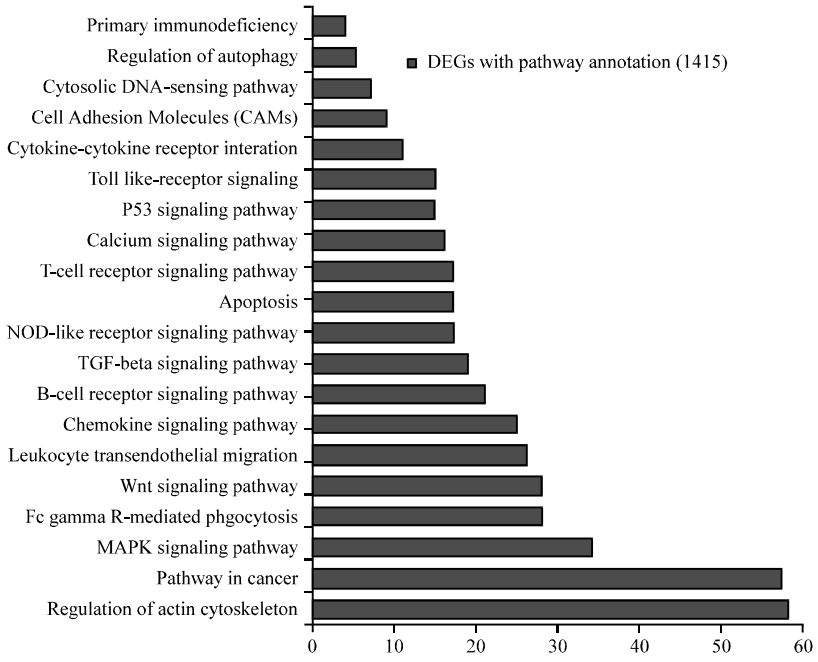

Fig. 7: Significant pathway categories. It had a p-value of <0.05 and an FDR of <0.05

capillary (microtubules) and the middle silk (intermediate filaments), dynamic distribution in cell (Chen *et al*., 2007). The GO indicated that the cell part is playing the vital role in virus's course of infection, 9542 genes can matched the libraries. The can translated to the host cell's skeleton system. In NDV infection host's RNA synthesis period, RNP (ribose nucleoalbumin compound) can assembled with the cell skeleton union, indicate that the cell skeleton ingredient is related with virus's RNA synthesizes (Hamaguchi *et al*., 1985). Cell part played the vital role in virus's invasion, the duplication, infect and bud processes.

NF-κB is a regulator of nuclear gene transcription factor, regulates many biologically active mediators such as cytokines, chemotactic factors, adhesion molecules, gene transcription and expression. NF-κB can activate and inhibited the apoptosis. NF-κB as an anti-apoptotic material, had already been confirmed in macrophage apoptosis induced by FasL NF-κB. It can reduce the incidence of apoptosis and macrophage apoptosis in the processing of the NF-κB inhibitor (Lu *et al*., 2002). The research showed that many genes found in I-kappaB kinase/NF-kappaB cascade.

TGFβ signaling pathway not only played an important role in cell and organism growth and development process, also closed associated with human tumorigenesis development. TGFβ can show anomalies if any component, in the signal transduction pathways TGFβ signal transduction, become disorder, resulting in tumor (Su *et al*., 2010). NDV applied to inhibit tumor clinically. The pathway analysis showed that TGFβ signaling pathway existed in NDV-YC infected DF-1 cells.

CONCLUSION

Deep-sequencing analysis of DF-1 cell transcriptome response to infection with newcastle disease virus of duck make further understand that NDV the pathogenesis mechanism as well as NDV come from waterfowl infected cell's essence. It has provided the experiment basis.

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REFERENCES

Alexander, D.J. and W.H. Allan, 1974. Newcastle disease virus pathotypes. Avian Pathol., 3: 269-278.

Audic, S. and J.M. Claverie, 1997. The significance of digital gene expression profiles. Genome Res., 7: 986-995.

Benjamini, Y. and D. Yekutieli, 2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat., 29: 1165-1188.

Cai, S., J. Li, M.T. Wong, P. Jiao and H. Fan *et al*., 2011. Genetic characterization and evolutionary analysis of 4 Newcastle disease virus isolate full genomes from waterbirds in South China during 2003-2007. Vet. Microbiol., 152: 46-54.

Chen, C., J. Jin, M. Rubin, L. Huang and T. Sturgeon *et al*., 2007. Association of gag multimers with filamentous actin during equine infectious anemia virus assembly. Current HIV Res., 5: 315-323.

Chu, H.P., G. Snell, D.J. Alexander and G.C. Schild, 1982. A single radial immunodiffusion test for antibodies to Newcastle disease virus. Avian Pathol., 11: 227-234.

Cloonan, N. and S.M. Grimmond, 2008. Transcriptome content and dynamics at single-nucleotide resolution. Genome Biol., 9: 234-234.

Collins, M.S., D.J. Alexander, S. Brockman, P.A. Kemp and R.J. Manvell, 1989. Evaluation of mouse monoclonal antibodies raised against an isolate of the variant avian paramyxovirus type 1 responsible for the current panzootic in pigeons. Arch Virol., 104: 53-61.

Eisen, M.B., P.T. Spellman, P.O. Brown and D. Botstein, 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA., 95: 14863-14868.

Glazov, E.A., P.A. Cottee, W.C. Barris, R.J. Moore, B.P. Dalrymple and M.L. Tizard, 2008. A microRNA catalog of the developing chicken embryo identified by a deep sequencing approach. Genome Res., 18: 957-964.

Hamaguchi, M., K. Nishikawa, T. Toyoda, T. Yoshida, T. Hanaichi and Y. Nagai, 1985. Transcriptive complex of Newcastle disease virus II. structural and functional assembly associated with the cytoskeletal framework. Virology, 147: 295-308.

Hegedus, Z., A. Zakrzewska, V.C. A Goston, A. Ordas and R.A. Cz, 2009. Deep sequencing of the zebrafish transcriptome response to mycobacterium infection. Mol. Immunol., 46: 2918-2930.

Hoen, P.A.C., Y. Ariyurek, H.H. Thygesen, E. Vreugdenhil and R.H.A.M. Vossen *et al*., 2008. Deep sequencing-based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms. Nucl. Acids Res., 36: e141-e141.

Hussein, H.A., A.A. El-Sanousi, A.A. Youssif, M.A. Shalaby, M.S. Saber and I.M. Reda, 2005. Sequence analysis of fusion and matrix protein genes of the velogenic viscerotropic newcastle disease virus egyptian strain sr/76. Int. J. Virol., 1: 38-38.

Kanehisa, M., S. Goto, S. Kawashima, Y. Okuno and M. Hattori, 2004. The KEGG resource for deciphering the genome. Nucl. Acids Res., 32: 277-280.

Lu, B., L. Wang, D. Medan, D. Toledo and C. Huang *et al*., 2002. Regulation of Fas (CD95)-induced apoptosis by nuclear factor-kappaB and tumor necrosis factor-alpha in macrophages. Am. J. Physiol. Cell Physiol., 283: 831-838.

Miller, P.J., E.L. Decanini and C.L. Afonso, 2010. Newcastle disease: Evolution of genotypes and the related diagnostic challenges. Infect. Genet Evol., 10: 26-35.

Morozova, O. and M.A. Marra, 2008. Applications of next-generation sequencing technologies in functional genomics. Genomics, 92: 255-264.

Morrissy, A.S., R.D. Morin, A. Delaney, T. Zeng and H. McDonald *et al*., 2009. Next-generation tag sequencing for cancer gene expression profiling. Genome Res., 19: 1825-1835.

OIE, 2009. Newcastle disease. In World Organisation for Animal Health.

Ravindra, P.V., A.K. Tiwari, B. Ratta, U. Chaturvedi, S.K. Palia and R.S. Chauhan, 2009. Newcastle disease virus-induced cytopathic effect in infected cells is caused due to apoptosis. Virus Res., 141: 13-20.

Saldanha, A.J., 2004. Java treeview-extensible visualization of microarray data. Bioinformatics, 20: 3246-3248.

Su, E., X. Han and G. Jiang, 2010. The transforming growth factor beta 1/SMAD signaling pathway involved in human chronic myeloid leukemia. Tumori, 96: 659-666.

Wang, Z., M. Gerstein and M. Snyder, 2009. RNA-Seq: A revolutionary tool for transcriptomics. Nat. Rev. Genet., 10: 57-63.

Wilhelm, B.T., S. Marguerat, S. Watt, F. Schubert and V. Wood *et al*., 2008. Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution. Nature, 453: 1239-1243.

Xiao, S., J. Jia, D. Mo, Q. Wang and L. Qin, 2010. Understanding PRRSV infection in porcine lung based on genome-wide transcriptome response identified by deep sequencing. PLoS One, 5: e11377 10.1371/journal.pone.0011377.

Yi, M., J. Horton, J. Cohen, H. Hobbs and R. Stephens, 2006. WholePathwayScope: A comprehensive pathway-based analysis tool for high-throughput data. Biol. Med. Chem. Bioinformatics, 7: 30-30.