Inspection of C-type lectin superfamily expression profile in chicken and mouse dendritic cells

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Abstract

C-type lectin superfamily is a cell surface molecule family well-recognized for its role in inducing and tailoring dendritic cell (DC) immune response. Despite its importance in DC targeting and activation, knowledge about C-type lectin family expression in chicken DC is still limited, and thus hinders its utilization for poultry DC vaccine development. In this study, expressions of C-type lectin orthologous gene among available chicken and mouse DC subsets were determined for expression levels and differential expression patterns. Leukocyte microarray datasets and bone marrow-derived DC mRNA-sequencing datasets were pre-processed prior to observation and comparison. Data virtualization revealed variation in C-type lectin gene expressions among the DC subsets, while the differential and relative expression results manifested PLA2R1 and CLEC16A as candidate genes for their future exploration at molecular and functional levels (FDR<0.001 when compared to lymphocytes, and relative expression level ≥ 0). Of note, a step-by-step procedure to extract novel C-type lectin gene candidates in chicken DC by cross-species comparative expression profiling analysis was illustrated in this study. Based on the same process, a future study is planned to apply the same procedures to investigate other gene families’ potential for DC targeting and activation in chicken.

Keywords: C-type lectin superfamily, dendritic cell, microarray, mRNA-sequencing

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Introduction

Dendritic cell (DC) is a professional antigen presenting cell (APC) with exquisite capacity to tailor specific immune response to antigen (Ag). DC is among one of the most diversified immune cells, of which each DC subset possesses its unique features and immunoproperties (Naik et al., 2007). Due to the key role of DC in initiating T lymphocyte adaptive immunity, DC is considered as a potential target for immunization by both facilitating Ag entry and cell activation. It is, thus, no surprise that such concept has become a popular strategy for vaccinations over the past two decades (Kreutz et al., 2013). Excitingly, discovery of DC in chicken also kick-starts such inspiration in poultry industry immunization (Wu and Kaiser, 2011).

DC biology is mostly studied in mouse model. Mouse DC subsets present in neutral state prior to immune response include type 1 interferon-producing plasmacytoid DC (pDC), lymphoid tissue-resident conventional DC (cDC) and migratory subsets such as Langerhans cell (LC) (Naik et al., 2007). Each mouse DC subset possesses its own unique phenotypic features; however, is distinguishable from other APCs majorly by cell surface Cd11c expression. Most DC subsets express considerable amount of Cd11c, except for pDC and LC, which assert low Cd11b expression. For lymphoid tissue-resident cDCs, the cells can be further classified into major Cd11b+cDC (1bDC) and CD8+cDC (cd8aDC) subsets (Lemos et al., 2003). More mouse DC subsets can be developed by inflammation or even generated ex vivo, such as the monocytically-derived DC (diff_mdDC) and bone marrow-derived DC (diff_bmDC). On the other hand, little is still known about chicken DC categorization. Probable chicken LC was identified beforehand in chicken skin (Igyarto et al., 2006), while in vitro diff_bm_DC-like cell was obtained later from culture of bone marrow cell in the presence of GM-CSF and IL-4 (Wu et al., 2010). Recently, the very first chicken lymphoid tissue-resident cDC was characterized in vitro based on its cDC-like morphology (Wu and Kaiser, 2011).

DC is regarded as an attractive target for immunization. With an aim to induce potent T cell response, most DC vaccines are usually designed to target and stimulate Pattern Recognition Receptors (PRRs) on DC surface. Among PRRs members, C-type lectin superfamily is well-recognized for such application. It is, therefore, not surprising that numerous C-type lectin molecules on mouse and human DCs have already been explored for clinical benefits (Geijtenbeek and Gringhuis, 2009). In contrast, C-type lectin expression on chicken DC is still poorly characterized (Wu and Kaiser, 2011). Besides the limitedly identified C-type lectin genes in chicken, insufficiency of specific antibody even further restricts the detection of target C-type lectin molecules interested by researchers. Compellingly, recent advance in chicken DC gene expression profiling (Vu Manh et al., 2014) could provide an alternative approach to surpass such limitation. With multiple probes on chicken microarray platform to detect target genes of interest, it is, thus, possible to screen several chicken C-type lectin transcripts with no need for definite detection system in the first place. To accomplish this objective, this study, therefore, performed a cross-species gene expression profiling analysis among mouse and chicken DC subsets. With the study’s outcome, novel C-type lectin candidate genes in chicken DC were successfully identified for further exploration at molecular and function levels.

Materials and Methods

Computer machine and operating system: Two computer machines with Ubuntu 14.04 LTS operating system (OS) were required. The server machine used for both microarray and RNA-seq data pre-processing had 32 CPU (Intel® Itanium series 9000 1.6-GHz) and 64 GB of RAM with 10 TB hard-drive space. For other analyses, a person machine with 2 CPU (Intel® Core™ i5-600), 16 GB of RAM with 10 GB hard-drive space was used.

R environment: R environment for statistical computing and graphics is freely available (http://www.r-project.org/). The R version applied for the study was 3.2.3 for linux-gnu (64-bit).

Leukocyte microarray datasets: Raw microarray expression CEL files and RNA-sequencing SRA files of leukocytes acquired from chicken (Gallus gallus) and mouse (Mus musculus) were retrieved from ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) to put together a total of 140 samples (15 chicken samples and 125 mouse samples). Cell type abbreviation is described in Table 1, and CEL file names of all samples used in the current study are also provided (Supplementary Tables 1 and 2) (further details are available in ArrayExpress). According to cell preparation protocols, all cell samples were in resting state.

Dendritic cell RNA-sequencing datasets: RNA-sequencing SRA files of chicken (Gallus gallus) and mouse (Mus musculus) bone marrow-derived DCs (diff_bm DCs) were retrieved from Sequence Read Archive (SRA), 1 for chicken and 2 for mouse samples (http://www.ncbi.nlm.nih.gov/sra/?term=). Genome files and genome index files were available on Ensembl genome browser (Table 2). According to cell preparation protocols, all diff_bm DC samples were in resting state.

Preparation for microarray data: Chicken and mouse raw microarray data were pre-processed and determined for their quality. For mouse data, the pre-processing was performed as described in our previous report (Chokeshai-u-saha et al., 2012; Chokeshai-u-saha and Sananmuang, 2014). For chicken data, ‘Normexp’ background correction and quantile normalization were applied (Diboun et al., 2006). To correct technical variation among the pre-processed mouse datasets, Bias correction method was implemented with the pre-processed data (Ekland and Szallasi, 2008). Quality metric reports of both chicken and mouse pre-processed datasets were produced and
assessed (Chokeshaiusa et al., 2015). Pooling of scaled pre-processed expression profiling datasets of chicken and mouse datasets was performed based on their orthologous genes using our previously reported methodology (Chokeshaiusa et al., 2015).

**Preparation for mRNA-sequencing data:** mRNA-sequencing paired-end reads were pre-processed as follows. Adapter trimming was performed by adaptive quality trimming approach (MAXINFO), and reads with adequate complexity (DUST score > 70) and length (≥ 53 bases) were selected using Trimmomatic (Bolger et al., 2014) and PRINSEQ software, accordingly. The pre-processed reads were subsequently aligned onto genomes corresponding to the species of samples with STAR spliced aligner (Dobin et al., 2013). Alignment output files were converted to bam files by Picard tool. Count reads per genes and Fragments Per Kilobase per Million mapped reads (FPKMs) were generated by HTSeq (Anders et al., 2015) and Cufflinks (Trapnell et al., 2012) software, respectively.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Abbreviations and their corresponding cell types</th>
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<tr>
<td><strong>Species</strong></td>
<td><strong>Abbreviation</strong></td>
</tr>
<tr>
<td><em>Gallus gallus</em> (chicken)</td>
<td>spl_B</td>
</tr>
<tr>
<td></td>
<td>spl_cDC</td>
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<tr>
<td></td>
<td>spl_Ma</td>
</tr>
<tr>
<td></td>
<td>spl_T</td>
</tr>
<tr>
<td><em>Mus musculus</em> (mouse)</td>
<td>diff_bm_DC</td>
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<td></td>
<td>diff_bm_Ma</td>
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<tr>
<td></td>
<td>lymph_cDC</td>
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<tr>
<td></td>
<td>pc_11bDC</td>
</tr>
<tr>
<td></td>
<td>pt_Ma</td>
</tr>
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<td></td>
<td>spl_B</td>
</tr>
<tr>
<td></td>
<td>spl_cd11bDC</td>
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<td></td>
<td>spl_cd8aDC</td>
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<td></td>
<td>spl_cDC</td>
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<td></td>
<td>spl_pDC</td>
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<td></td>
<td>spl_T</td>
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**Table 2** RNA-sequencing data files used in the study

<table>
<thead>
<tr>
<th>File type</th>
<th>File name</th>
</tr>
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<tr>
<td>Sample</td>
<td>ERR652849</td>
</tr>
<tr>
<td>Genome</td>
<td><em>Gallus_gallus.Galga4.dna_sm.toplevel.fa.gz</em></td>
</tr>
<tr>
<td></td>
<td><em>Mus_musculus.GRCm38.dna_sm.toplevel.fa.gz</em></td>
</tr>
<tr>
<td>Genome index</td>
<td><em>Gallus_gallus.Galga4.79.gtf.gz</em></td>
</tr>
<tr>
<td></td>
<td><em>Mus_musculus.GRCm38.82.gtf.gz</em></td>
</tr>
</tbody>
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**Differential gene expression analysis of DC:** Local Pooled Error method was implemented for significant analysis (Jain et al., 2003; Murie and Nadon, 2008; Chokeshai-u-saha et al., 2012). Each of the DC subsets was coupled with each of B lymphocyte, T lymphocyte, and macrophage populations. Splenic B (spl_B) and T lymphocytes (spl_T) were selected for the test. For macrophages, mouse bone-marrow derived macrophage (diff_bm_M) and chicken splenic macrophage (spl_M) were chosen. For every test conducted, each DC subset was regarded as reference cell population. The test was assigned to each pairing (DC vs spl_B, DC vs spl_T and DC vs diff_bm_M/spl_M), and acquired p-value was adjusted by resampling based False Discovery Rate (FDR) adjustment for 1,000 iterations.

**Virtualization of microarray data and mRNA-sequencing data:** Principal Component Analysis (PCA) and Hierarchical clustering of C-type lectin orthologous genes were carried out on the pre-processed microarray data. A 3-dimensional plot was drawn from principal component 1 (PC1), PC2 and PC3 scores. A heatmap was drawn from the scaled microarray expression values similar to our previous report (Chokeshaiusa et al., 2015). A categorical bubble plot was drawn to observe differential expression results of DC. Bar plots were drawn from the scaled microarray expression value of each DC subsets and from the scaled FPKMs (processed from mRNA-seq data) of C-type lectin superfamily gene acquired from both chicken and mouse.
**Results**

Principle component analysis revealed separate clusters of DCs from other lymphocyte populations:

The pre-processed microarray expression profiling datasets of chicken and mouse leukocytes samples were analyzed by the Principle component analysis (PCA). The virtualization of PCA was plotted with the 3-dimensional projection using the principal component (PC) 1, PC3, and PC2, accordingly (Fig 1). Of note, PCA resulted in clear separation of DCs from other lymphocyte populations in both species. Chicken DCs were clearly assembled from other leukocyte cell types, while mouse DC subsets were all intact with the macrophage populations (Fig 1).

![Figure 1](image-url)

**Figure 1** Principle Component Analysis (PCA) results of leukocyte samples acquired from chicken (1A) and mouse (1B). The acquired PCA results of chicken (1A) and mouse (1B) are presented in 3-dimensional plots. The x, y and z axes denote the value of principal component 1 (PC1), PC3, and PC2, accordingly. Each dot in the figure represents one individual leukocyte sample. The dot color legends presented adjacent to the plots indicate the sample cell types of which their abbreviations are described in Table 1. Very clear clusters of splenic T lymphocytes (blue sphere), B lymphocytes (pink sphere), macrophages (green sphere) and dendritic cells (orange sphere) are presented in the chicken PCA results (1A). Clear clusters of splenic T lymphocytes (blue sphere) and B lymphocytes (pink sphere) are also presented in the mouse PCA results. However, clusters of each macrophage and dendritic cell type are not clearly outlined. Closed relationships among the mouse macrophage and dendritic cell populations are presented with a clear cluster (black sphere) separated from the lymphocyte population (2A).
Figure 2  Heatmap of C-type lectin superfamily gene across mouse and chicken microarray platforms. The heatmap row represents symbols of orthologous genes available between the two species. The row represents genes clustered by Pearson correlation distance (1-Correlation coefficient) with complete linkage, while the column represents cell samples clustered according to their original cell types. For further gene symbol description, the Mouse Genome Informatics (MGI) gene symbol begins with an upper-case letter, followed by all lower-case letters (e.g. Cd69 and Clec18a). The chicken gene symbol is in all uppercase (e.g. CD69 and CLEC18A). Since some probes provided by the chicken microarray platform were actually designed from human transcripts, an ‘h’ letter was added at the beginning of all Hugo Gene Nomenclature Committee (HGNC) symbols achieved from human gene (e.g. (h)CLEC4D and (h)CLEC4E).
Figure 3  Categorical bubble map of C-type lectin superfamily gene comparisons between each DC subset (row) and each lymphocyte/macrophage population (column). The B and T lymphocytes applied for each pairwise comparison were from splenic B lymphocytes (spl_B) and splenic T lymphocytes (spl_T) in both species. For macrophage populations, mouse differentiated bone-marrow macrophage (diff_bm_Ma) and chicken splenic macrophage (spl_Ma) were used. All cell type abbreviations are described in Table 1. For legend description, the red dot attributes to higher gene expression in dendritic cell. The blue dot attributes to higher gene expression in the compared cell type. The color intensity corresponds to the acquired resampling FDR adjusted p-value, while its surface area is proportional to the acquired fold-change in expression level.
Figure 4  Scaled C-type lectin superfamily gene expression values acquired among DC subsets. The microarray expression values of all available C-type lectin genes were normalized by median-absolute deviation scaling to enable expression comparison between chicken and mouse DC subsets (4A). In order to perform within gene family expression observation, FPKM read values of CLEC16A, CLEC3B, MRC2 and PLA2R1 genes acquired from mouse and chicken bone marrow-derived dendritic cell RNA sequencing data were scaled by the within gene family expression level and plotted (4B).
Probes to detect (h)CLEC4D, (h)CLEC4E, (h)CD302 and Clec4a2 gene expressions in chicken microarray platform were ambiguously specific to chicken transcripts: To observe C-type lectin orthologous gene expression profiling across mouse and chicken leukocyte populations, the genes of C-type lectin superfamily available in each of the species microarray platforms were taken into account (Fig 2). Of note, several probes to detect C-type lectin genes in the chicken platform were designed from human/mouse transcripts, and as a result inherited ambiguous specificity to chicken transcripts (CLEC4D, CLEC4E and CD302 from human and Clec4a2 from mouse). Only probes specific to CD69, CLEC16A, CLEC18A, CLEC3B, LEPR, MRC2 and PLA2R1 were authentic to detect chicken orthologous genes. Variation in the expression pattern of C-type lectin genes among the mouse and chicken leukocyte cell types were clearly noticeable (Fig 2). Cd69 (mouse)/Cd69 (chicken) expression was concentrated in lymphocyte populations. Clec16a (mouse) was slightly concentrated in diffuse bm DC, pc_1lbDC and lymph_cDC, while the CLEC16A orthologous gene (chicken) was clearly concentrated in spl_cDC. Random expressions of Clec3b and Pla2r1 among the mouse leukocytes were obvious, whereas their chicken orthologous genes (CLEC3B and PLA2R1) were concentrated in myeloid populations (spl_DC and spl_Ma) (Fig 2).

Differential expression analysis highlighted CLEC3B, CLEC16A and PLA2R1 with unique FDR and FC patterns in chicken spl_DC, but not different from other mouse DC subsets: Differential C-type lectin gene expression results of the chicken and mouse DC subsets were reported based on acquired False Discovery Rate (FDR) and Fold-Change (FC) (Fig 3). All DC subsets expressed Cd69 (mouse)/Cd69 (chicken) lower than lymphocytes. Chicken spl_cDC differential expressions of Cd302 displayed reversed FDR, and of Clec4a2, Clec4d and (h)CLEC4E presented very low FC (FC<0.5) when compared to the mouse DC subsets. Most of the other mouse-chicken orthologous genes demonstrated weak variations in the FDR and/or FC patterns, except for CLEC3B and PLA2R1, which displayed distinctive FDR and FC patterns in chicken spl_cDC when compared to other mouse DC subsets (Fig 3).

Chicken spl_cDC expressed PLA2R1 above average leukocyte expression level, and chicken diff_bm_DC expressed PLA2R1 above within C-type lectin family level: Considering the microarray datasets, C-type lectin genes among the mouse and chicken DC subsets were determined for their expressions relative to all leukocyte expression levels. Varied expression levels among the DC subsets were clear, and several C-type lectin genes were found to express above average levels among the mouse DC subsets. On the contrary, chicken spl_cDC only expressed PLA2R1 higher than average (Fig 4A). Relative expression of C-type lectin orthologous gene within gene family was further investigated in the mRNA-seq datasets of mouse and chicken diff_bm_DC. The relative mRNA-seq FPKM value of Clec16a/CLEC16A was above average in both species, while different trends in Mrc2/MRC2 and Pla2r1/PLA2R1 expressions were presented between them. Of note, chicken diff_bm_DC showed lower MRC2 than average, but PLA2R1 equal to average level (Fig 4B).

Discussion

A comparative expression profiling analysis of C-type lectin gene superfamily between mouse and chicken DCs was demonstrated in this study. Despite bias corrected by the procedures (Chokeshaiusaha et al., 2015), some concerns should be noted prior to result interpretation. The leukocyte datasets in this study were derived from separate experiments with possible inconsistency in cell preparations, which could result in biological variations among the samples within the same cell type (Fig 2). Besides, comparable orthologous gene number between the two species was restricted by the available probe number. In chicken microarray platform, utilization of probes from human or mouse transcripts could result in non-functional, mis-annotated or even mis-orthologous probes. Since aberrant expressions of such probes were also implied in our study, the interpretation of (h)CLEC4D, (h)CLEC4E, (h)CD302 and Clec4a2 was, therefore, excluded in the chicken platform (Figs 2 and 3). It should also be noted that only the splenic conventional DC (spl_cDC) microarray data were available in chicken. Generalization of the acquired result to other chicken DC subsets must be cautiously implied. Finally, very limited mRNA-seq datasets were obtained, as a result they were only conserved as supplements for acquired microarray results.

To our knowledge, none of the C-type lectin orthologous gene presented in this study has clearly been explored for its biological role in chicken DC. Moreover, only Cd69/Cd69, Clec16a/CLEC16A and Pla2r1/PLA2R1 have contributed their functional evidences in mouse or human DCs (Table 3). Since Cd69 upregulation is common during DC activation in human and mouse (Lamana et al., 2011), lower Cd69/Cd69 expression in all DC subsets than lymphocytes were expected (Figs 2 and 3). Prominent CLEC16A expression in chicken spl_cDC when compared to Clec16a expression in other mouse DC subsets isolated from spleen (mouse spl_cDC, mouse spl_cd11bDC, and mouse spl_cdb8aDC) was demonstrated (Figs 2, 3, and 4A), and the within C-type lectin gene family expression of diff_bm_DC result (Fig 4B) also showed gene expression above average level in both species. CLEC16A function in chicken DC has still been unexplored, however its contribution in Major histocompatibility class II (MHC class II) formation was evidenced in human DC (van Luijn et al., 2015). Noteworthy, the CLEC16A expression in chicken DC could imply its high expression at molecular level, and thus possibly be exploited for chicken DC targeting.

CLEC3B and PLA2R1 appeared to show unique differential expression patterns in chicken spl_cDC when compared to their orthologous gene expressions in the mouse DC subsets (Fig 3). Unlike CLEC3B, all chicken spl_cDC and diff_bm_DC
expressed PLA2R1 higher than the average leukocyte level and equal to the within gene family average level, respectively (Figs 4A and 4B). In human DC, PLA2R1 is responsible for type I receptor of phospholipase A2, an enzyme capable of promoting DC maturation (Perrin-Cocon et al., 2004). Compellingly, the unexplored role of phospholipase A2 in chicken DC along with its prominent PLA2R1 expression in this study, therefore, make PLA2R1 of interest for further investigation into its molecular function.

<table>
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<th>Ortholog</th>
<th>Encoded protein</th>
<th>Evidenced role in dendritic cell</th>
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<tbody>
<tr>
<td>CD69</td>
<td>Clec69</td>
<td>Early inducible leukocyte activation receptor (Lamana et al., 2011)</td>
</tr>
<tr>
<td>CLEC3B</td>
<td>Clec3b</td>
<td>Unexplored</td>
</tr>
<tr>
<td>CLEC16A</td>
<td>Clec16a</td>
<td>Essential for Major histocompatibility class II (MHC class II) formation in human DC (van Luijn et al., 2015)</td>
</tr>
<tr>
<td>MRC2</td>
<td>Mrc2</td>
<td>Unexplored</td>
</tr>
<tr>
<td>PLA2R1</td>
<td>Pla2r1</td>
<td>Might play a role in cell maturation process through phospholipase A2 signaling (Perrin-Cocon et al., 2004)</td>
</tr>
</tbody>
</table>

In conclusion, the C-type lectin gene expression profiling analysis between chicken and mouse DC subsets in this study implied CLEC16A and PLA2R1 as compelling candidates for function exploration in chicken DCs. Obviously, the limited number of informative probe for orthologous gene detection should be regarded as the major limitation to study large-scale C-type lectin superfamily in this study. In order to achieve more extensive novel candidate genes, not only inclusion of human DC subsets is planned in our future study, but also consideration of all other important gene families responsible for Pattern Recognition Receptors (PRRs) expression.

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**References**


บทคัดย่อ

การศึกษาโปรไฟล์การแสดงออกของยีนในวงศ์ซีไทป์เลคตินซุเปอร์แฟมิลีของเดนไดรติคเซลล์ในไก่

เปรียบเทียบกับหนูทดลอง

การ โภชนาศาสตร์† รุ่งโรจน์ ธนาวงษ์นุเวช‡ เดนิส พูทิเยร์§ แคเธอรีน เหงียน∥

โมเลกุลในวงศ์ซีไทป์เลคตินเป็นโมเลกุลที่มีบทบาทสำคัญที่เดนไดรติคเซลล์ใช้ในการจับกันเชื้อและควบคุมกระบวนการตอบสนองทางภูมิคุ้มกันของเซลล์ด้วยเหตุนี้โมเลกุลในวงศ์ซีไทป์เลคตินจึงเป็นเป้าหมายสำคัญสำหรับการศึกษาอย่างละเอียด รวมถึงการศึกษาการทำงานของโมเลกุลในวงศ์ซีไทป์เลคตินในเซลล์ต่าง ๆ ด้วยเหตุนี้การศึกษาครั้งนี้จึงทำให้ศึกษาได้ในโมเลกุล การแสดงออกของยีนในวงศ์ซีไทป์เลคตินในแต่ละประเภทของเดนไดรติคเซลล์ของไก่และหนู โดยอาศัยข้อมูลไมโครอะเรย์และข้อมูลเอ็มอาร์เอ็นเอซี เควนซิ่ง และนำข้อมูลดังกล่าวผ่านกระบวนการจัดเตรียมอย่างเหมาะสมก่อนการวิเคราะห์ ผลจากการวิเคราะห์ข้อมูลแสดงให้เห็นว่าโมเลกุลแข็งแกร่งที่มีการการแสดงออกในวงศ์ซีไทป์เลคตินที่แตกต่างกัน โดยผลการเปรียบเทียบระดับการแสดงออกของยีนแสดงให้เห็นว่ายีน PLA2R1 และ CLEC16A เป็นยีนที่มีระดับการแสดงออกต่ำในโมเลกุลในวงศ์ซีไทป์เลคตินของกบ ซึ่งแตกต่างจากในโมเลกุลในวงศ์ซีไทป์เลคตินของหนู (FDR<0.001 เมื่อเทียบกับกบ) และมีระดับการแสดงออกสูงกว่าค่าเฉลี่ย ปัจจุบันของเจ้าภาพที่เหมาะสมสำหรับการศึกษาเพื่อที่จะยืนยันระดับการแสดงออกในโมเลกุล รวมถึงหน้าที่ของยีน ต่อไป โดยกระบวนการศึกษาที่เน้นจากการศึกษาครั้งนี้สามารถนำไปประยุกต์ใช้ในการศึกษาอย่างมีนัยสำคัญ ๆ ที่มีบทบาทสำคัญในโมเลกุลในวงศ์ซีไทป์เลคตินของไก่ในอนาคต

คำสำคัญ: ซีไทป์เลคติน เดนไดรติคเซลล์ ไมโครอะเรย์ เอ็มอาร์เอ็นเอซีควนซิ่ง

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