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Original Article

Transcriptional Profiling of Age-Associated Gene Expression Changes in Human Circulatory CD1c$^+$ Myeloid Dendritic Cell Subset

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Abstract

Immune dysfunction is a hallmark of aging and is thought to be responsible for the age-associated diseases. Dendritic cells (DCs) of the immune system function as initiators and regulators of the immune responses. Recent studies have highlighted the division of labor between various DC subsets. CD1c$^+$ DC subset has emerged as a major inducer of CD4 T cell response. There is a scarcity of information regarding the age-associated changes in the functions of DC subsets in the elderly. Here, we investigated the changes in transcriptional profile of CD1c$^+$ DC subset from healthy aged and young individuals using RNA sequencing. Our results suggest that majority of the genes in DCs are upregulated with age. Glucose transport, GPCR, and potassium channel genes are all upregulated in DCs from aged as compared to young indicating an enhanced activation state of DCs from aged individuals. The expression of histones, small nucleolar RNA H/ACA box (SNORA) and small nucleolar RNA C/D/box (SNORD), and long non-coding RNA (lncRNA) is also substantially upregulated in the DCs from aged. In contrast, the antigen-presenting and energy generating pathways are downregulated. In summary, DCs from aged subjects display an activated state coupled with reduced antigen presentation which may be responsible for age-associate immune dysfunction.

Keywords: Myeloid dendritic cells, RNA-seq, Aging

Dendritic cells (DCs) are the major antigen-presenting cells of the body. DCs are the primary responders to a threat as well as play a critical role in limiting inflammation to self and harmless antigens in the periphery and in the mucosa. The initiation of adaptive immunity relies on DCs, which are professional antigen-presenting cells (APCs) with the capacity to activate naïve T cells (1,2). DCs by virtue of their cytokine secretion also dictate the polarization of Th cell responses (3,4). DCs have been divided into two major subsets: plasmacytoid DCs (pDCs) and myeloid DCs (mDCs). pDCs are an important part of antiviral defense because of their ability to produce copious amounts of type I interferons very early and fast during an infection. mDCs represent the conventional DCs which are important for antigen presentation and T cell priming. mDCs in the blood can be divided into two major subsets CD1c and CD141$^+$ (5,6). mDCs comprise about 0.5–1.5% of human peripheral blood mononuclear cells (PBMCs) of which CD1c expression defines the most abundant mDC subset in peripheral blood. CD1c$^+$ mDCs are considered homologous to mice CD8α$^-$ DCs and are capable of secreting most inflammatory cytokines on stimulation (7). CD1c$^+$ mDC are also considered better stimulators of naïve CD4 T cells because they express high levels of mRNA for molecules associated with MHC class II processing. These include genes encoding for Ifi30 (GILT), HLA-DMA, and Cathepsin H (8). CD141$^+$ mDCs comprise only 0.1% of the PBMCs are considered better activators of CD8 T cells due to their ability to cross-present antigens (9–12). CD1c$^+$ mDCs are also able to cross-present antigen to CD8$^+$ T cells but not

as efficiently as CD141$^+$ mDCs (11,12). The mDC and pDC subsets also exist in the tissues. Thus, an understanding of DC function in the blood can give an indication of their functions in the tissues.

Advancing age has a profound effect on DC functions. Several studies have extensively investigated the alterations of DC functions induced by aging (13,14). Phagocytosis, migration, and cytokine secretion are all reported to be impaired in DCs from elderly individuals (13,14). Moreover, we (15) and others (16) have shown that DCs isolated from aged individuals exist in a semi-activated state. This semi-activated state in aged DCs is characterized by increased basal level of NF-κB activation coupled with increased low level of pro-inflammatory cytokine secretion even without infection. The secretion of type I and type III interferons in response to influenza is also reduced (17,18). While all these studies point towards a role of DCs in the immune dysregulation in aging, most studies were conducted utilizing human monocyte-derived DCs (MoDCs) as models for mDCs. Strikingly, there is a scarcity of information about how aging affects the functions of human mDC subsets in the blood and tissues due in part to their limited availability. This is especially important because recent studies have demonstrated that MoDCs express a gene pattern distinct from that of mDCs in circulation (6). Another equally important fact is that DC subsets from circulation populate the tissues where signals from the local milieu specializes their functions to suit the environment (19). This highlights the importance of understanding the effect of age on the mDC in the elderly where the subject is relatively unexplored.

Here, we compared the age-associated gene expression changes of the major mDC subset, the CD1c$^+$ mDCs in circulation between aged and young subjects in order to understand the role of this mDC subset in dysregulated immunity in the elderly.

Material and Methods

Blood Donors

Peripheral blood samples were obtained from healthy aged and young volunteers. The young donors were between 27 and 34 years of age. Healthy aged donors were between 61 and 70 years. Elderly subjects belong to middle-class socioeconomic status and are living independently. Subjects suffering from diseases such as diabetes, heart diseases, or those on long-term medications as well as those taking drugs that can affect the immune system were excluded from the study. Description of the cohort is provided in Supplementary Tables 1 and 2. This study was approved by the Institutional Review Board of the University of California, Irvine.

CD1c$^+$ DC Purification

DCs were purified from the PBMCs of young subjects by negative selection using DC enrichment kit (Stemcell technologies, Vancouver, Canada). Subsequent sorting was done to obtain pure CD1c$^+$ mDC population. The markers used for sorting were CD11c, CD123, and CD1c. Details are provided in Supplementary Figure 1.

RNA Sequencing

Complete transcriptome analysis of DCs isolated from three young and three old individuals were carried out (description of cohort in Supplementary Table 1). RNA isolation was performed using Qiagen RNeasy plus mini kit (Cat#74134) according to the manufacturer's instructions. The quantity of RNA was in picograms therefore SMARTer® Stranded Total RNA-Seq Kit t with Picogram input was used to construct the libraries (Clontech, Cat #634411). Libraries were quantified and normalized using the Library Quantification Kit from Kapa Biosystems and sequenced as paired-end 100 bp reads on the Illumina HiSeq 2500 platform. Library preparation and all transcriptome [High throughput sequencing (NGS) experiments were performed at UCI Genomic High Throughput Facility, GHTF (http://dmaf.biochem.uci.edu] using Illumina Genome Analyzer which generated about 10–20 million reads (30nt) from DCs derived from young and aged individuals.

Transcriptome Analysis Work Flow

Millions of reads for each of the DC cells were QC analyzed based on quality score distribution. Processed reads were mapped to reference genome (hg19) using Strand NGS v1.3 (Agilent; http://www.strand-ngs.com/) data analysis package. Mapped sequencing reads were analyzed. Briefly, deduplication was performed to remove duplicate reads generated by PCR amplification bias during library construction. Deduplicated sequencing reads were quantitated for expression levels of genes, exon partitions, and transcripts. This is done by counting the number of reads (raw reads) that map to gene or exon of interest. The raw counts were normalized using DESeq normalization method. Normalized counts are log transformed and baselined based on the mean expression levels of all samples (Strand NGS—RNA seq analysis).

Flow Cytometry

PBMCs from aged and young donors were stained with Lineage cocktail, HLADR, CD1c, and HLA-ABC (BD Biosciences) or CD200R (Miltenyi Biotech). Cells were gated on Lineage-, HLADR$^+$ CD1c$^+$. Expression of CD200R, HLA-ABC was determined on gated cells. Analysis was performed using Flow jo software. Description of cohort used for these experiments is provided in Supplementary Table 2.

Statistical Analysis

Statistical analysis for flow cytometry experiments was performed using GraphPad Prism (GraphPad Inc., San Diego, CA). Mann–Whitney test was used for the analysis. A p-value of $< .05$ was considered statistically significant. Statistical analysis for heat maps was performed using RNA seq analysis modules in Strand NGS v1.3. The values for heat maps are based on Audic Claverie (AC) test results with a false discovery rate (FDR) of $p < .05$.

Results

Gene Expression Differences Between CD1c$^+$ mDCs From Aged and Young Subjects

Paired end RNA sequencing (RNA-Seq) was performed to determine the gene expression differences between circulatory CD1c$^+$ DC from aged and young individuals. Sequencing reads were aligned to reference human genome (ie, hg19) using Bowtie algorithm in Strand NGS data analysis package (Agilent). Approximately 70–90% of mapped reads were aligned to protein coding regions (Supplementary Figure 2). Pooled analysis was performed using AC Test which pools together raw counts across three young DCs and raw counts across three old DCs separately and test the differences between young and old based on Poisson assumption on distribution of counts. Multiple Testing Correction: Benjamini Hochberg FDR of 0.05 and the maximum p value cut-off .05 was used. The AC test generated 9,682 gene differences between the two groups on multiple testing corrections (Benjamini Hochberg FDR, Supplementary

Table 1). The genes of AC test were used to determine transcripts with two or more fold change differences between the aged and the young groups. About 4,215 genes were differentially expressed with majority of the genes being upregulated in aged compared to young individuals (Figure 1A, Excel Supplementary File). Only 449 of the 4,215 total gene which displayed change were downregulated in DCs from aged subjects. Genes in aged subjects also displayed a wider distribution pattern (Figure 1B and C).

Validation of Genes Expression Changes Observed in RNA-seq at the Protein Level

To further confirm our RNA-seq data, the expression of three randomly chosen genes- CD200R1, HLADR, and HLA-ABC was compared on CD1c$^+$ mDCs from aged and young individuals using flow cytometry. CD200R1 is an inhibitory receptor which interacts with CD200 present on various cell types to regulate inflammatory responses (20). HLADR is Class II MHC required for antigen presentation to CD4 T cells. HLA-ABC is the Class I MHC required for activation of CD8$^+$ T cells (21). As is evident from Figure 2, the expression of CD200R was significantly upregulated ($p < .05$) while the expression of HLADR was significantly reduced ($p < .05$) in CD1c$^+$ mDCs from aged individuals compared to their young counterparts. The expression of HLA-ABC was comparable between the two groups. The change in protein expression confirmed the differences observed at the gene level.

Reduced Expression Antigen Presentation Genes in CD1c$^+$ mDC From Aged Individuals

Pathway analysis of the genes revealed significant downregulation of antigen presenting genes, HLA class II histocompatibility antigen DR beta 5 chain (HLA-DRB5), HLA Class II histocompatibility antigen DQ alpha 2 chain (HLA-DQA2), and Rab-interacting lysosomal protein (RILP) in DCs from aged individuals relative to young subjects (Figure 3). HLADQ is required to remove the CLIP molecules from MHC II complex to facilitate loading of antigenic peptides (22). HLAD-RB5 forms a dimer with the HLADR alpha chain and presents peptides to CD4 T cells (23). RILP plays a major role in trafficking of proteins in late endosomes (24). Reduced expression of these genes would suggest that antigen presenting capacity of CD1c$^+$ mDCs is compromised with age.

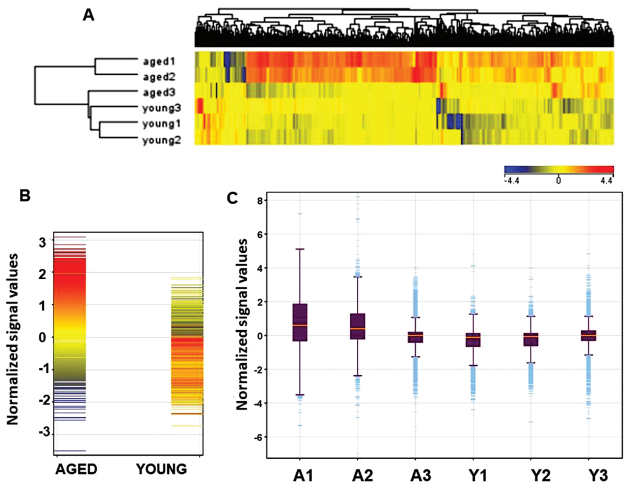

Figure 1. Overview of gene expression changes in CD1c$^+$ dendritic cells (DCs) from aged and young subjects. (**A**) Heat map depicts significant twofold changes in gene expression in aged and young DCs. (**B**) Plot depicts the cumulative gene distribution of DCs from aged and young. (**C**) Box plot depicts the gene distribution profile in individual aged and young samples. Range depicts log 2-fold changes in expression between CD1c$^+$ DCs from aged and young subjects. The values are based on Audic Claverie test results with a false discovery rate of $p < .05$.

Reduced Expression of Electron Transport Genes in DCs From Aged Individuals

Several genes present in the mitochondrial inner membrane UQCRH, NDUF-A1, A2, A8, S6 which are involved in mitochondrial electron transport chain to generate energy are down regulated in DCs from aged individuals (Supplementary Table 3) indicating alterations in mitochondria and the energy metabolism.

Enhanced Expression of Nutrient Transport Genes in DCs From Aged Subjects

One of the major pathways upregulated in DCs from aged subjects was the glucose transport pathway (Figure 4). Glucose is amongst primary source of energy for DCs. Glucose uptake involves non-concentrative glucose carriers of the GLUT family as well as Na$^+$-coupled glucose-carrier. The expression of GLUT5, SLC5A1/SGLT1, and SLC5A9/SGLT4, sugar transporters is upregulated in DCs from aged. The SLC5A1 gene, encodes the sodium/glucose co-transporter 1 (SGLT1) and is reported to be expressed in immune cells (25). SLC5A9/SGLT4, is also a Na$^+$-dependent glucose transporter (26,27). The expression of SLC2A5/GLUT-5, fructose transporter is

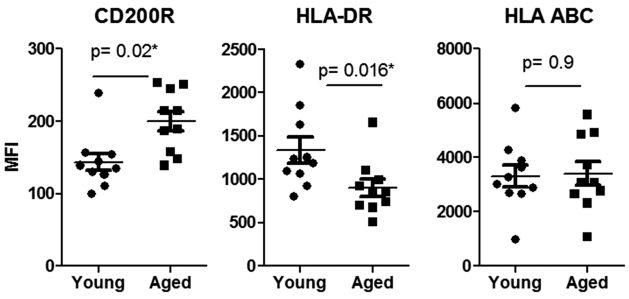

Figure 2. Validation of gene expression changes at the protein level. Three randomly chosen genes changes between aged and young were verified at protein level by flow cytometry. Graphs depict the changes median fluorescence intensity (MFI) in CD200R1, HLADR, and HLA-ABC. Data is Mean ± SE of 10 experiments from different donors. Mann–Whitney test p values depicted.

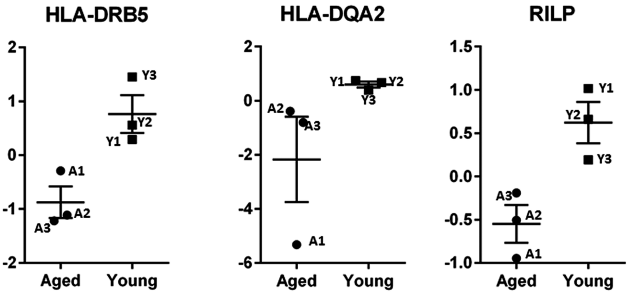

Figure 3. Reduced expression of antigen-presenting genes in DCs from aged subjects. Graphs depict the normalized expression values of genes HLA-DRB5, HLA-DQA2, and RILP from the RNA-seq. data present in Supplementary table 1.

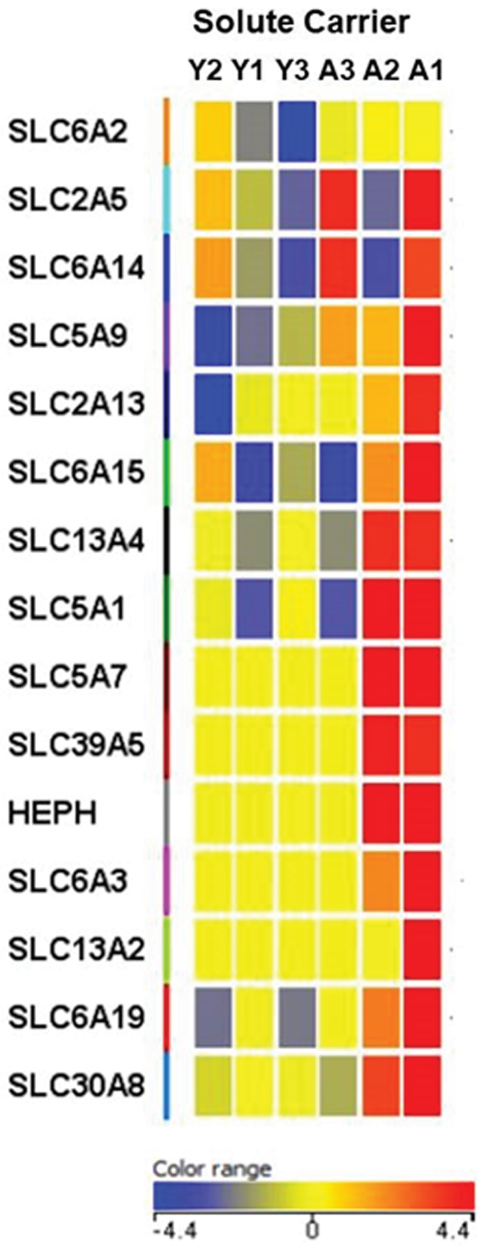

Figure 4. Enhanced expression of glucose-transport genes in dendritic cells (DCs) from aged subjects. Heat map depicts the expression of 15 genes in the solute carrier pathway which displayed significant changes in aged DCs compared to young DCs. Range depicts log 2 fold changes in expression between CD1c+ DCs from aged and young subjects. The values are based on AC test results with a FDR of $p < .05$.

also upregulated. In addition to glucose, expression of amino acid transporters is also upregulated in DCs from aged. The expression of SLC6A14 which transports all neutral amino acids as well as the cationic amino acids lysine and arginine and SLC6A19, a Na^+-dependent transporter of all neutral amino acids (26,27) is upregulated in DCs from aged as compared to young (Figure 4). SLC6A15 gene encodes for a sodium-dependent branched-chain amino acid transporter. Proline therefore has a high affinity for ***SLC6A15*** and is a precursor of glutamate synthesis (26,27). The expression of Zinc transporters SLC39A5, SLC30A8 are also upregulated in DCs from aged individuals (Figure 4). Thus, aging seems to induce significant metabolic changes in DCs.

The Expression of G-Protein Coupled Receptor (GPCR) Activity Genes and Potassium Channel Genes Is Upregulated in the DCs From Aged Individuals

The GPCR pathway genes were also significantly upregulated in the DCs from aged individuals verses the young (Figure 5A). Amongst the genes, almost 15% of the genes were the of chemokine receptors. Most of these receptors are considered inflammatory chemokine receptors which enhance the capacity of DCs to migrate towards inflamed tissues (28). For example, DCs migrate to inflamed tissues such as skin via upregulation of CCR6 (29,30). In addition to chemokine receptors, the expression of Sphingosine 1 receptors involved in migration, SIPR1, SIPR3, SIPR4, and SIPR5 is also upregulated on DCs from aged subjects.

Furthermore, one of the other pathways which displayed significant upregulation in aged subjects was the potassium channel pathway (Figure 5B). Majority of the genes in voltage-gated potassium channel family (Kv channels) are upregulated. Kv channels are also involved in cytokine production, maturation, and migration of DCs. Together, the enhanced expressions of these genes indicate that DCs from aged exhibit an activated state compared to DCs from young individuals.

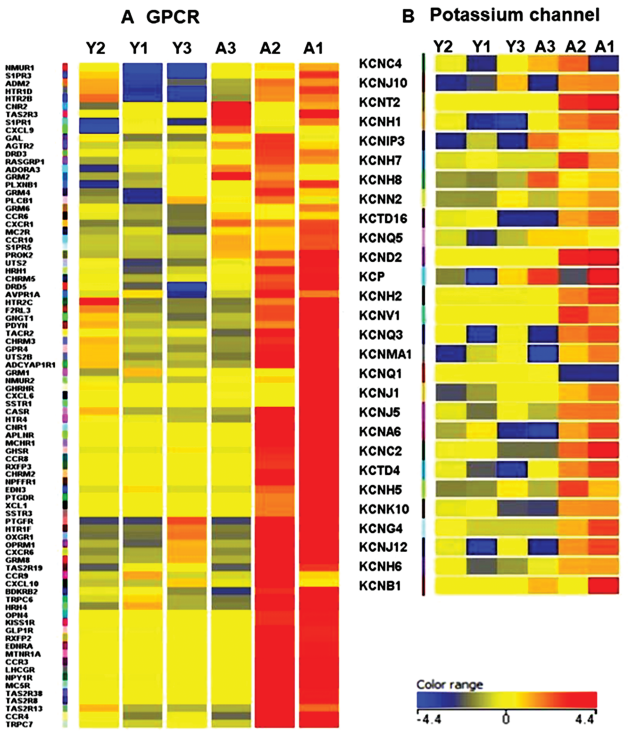

Figure 5. Enhanced expression of G-protein coupled receptor (GPCR) signaling and potassium channel genes in dendritic cells (DCs) from aged subjects. Heat map depicts the expression of (**A**) 88 genes in the GPCR signaling pathway; (**B**) 30 genes in potassium channel pathway which displayed significant changes in aged DCs compared to young DCs. Range depicts log 2 fold changes in expression between CD1c+ DCs from aged and young subjects. The values are based on AC test results with a FDR of $p < .05$.

Discussion

This study investigates the differences at the transcriptional level between circulatory CD1c$^+$ DCs from aged and young individuals. CD1c$^+$ DCs have emerged as major producers of IL-12p70 and IL-23 in response to extracellular and intracellular threats (31,32). Thus CD1c$^+$ DC possess the capacity to induce both Th1 and Th17 responses (31). Studies have also demonstrated that they can produce IL-10 to prime T regulatory responses (33). In addition to CD4, CD1c$^+$ DCs in blood and lymphoid organs have also been demonstrated to have the capacity to cross-present antigen to CD8 T cells and prime cytotoxic responses (32,34,35). The capacity to prime both CD4 and CD8 T cell responses represents a key difference in function of CD1c$^+$ DCs functions between mice and humans and highlights the importance of gaining an insight into age-associated changes in function of DC subsets in humans to design successful vaccines.

Our data indicates that DCs from aged individuals are deficient in the expression of antigen presenting molecules (Figure 3). Previous studies from our laboratory and others using monocyte-derived DCs had suggested that the antigen-presenting capacity of DCs is not affected with age. All these studies had been performed using allogenic culture and there is no report in humans examining the antigen presentation by other DC subsets using a syngeneic system. Influenza vaccination studies as well as the impaired response of elderly to infections suggest a defect in the immune system but the role of CD1c$^+$ DC subsets has not been examined. Few studies have examined the antigen presentation by various DC subsets in mice. A defect in CD8T cell priming by aged DCs has been reported in murine studies (36,37). Recent reports also demonstrate impairment in antigen cross-presentation by CD8$^+$ DC subset in aged mice (37,38). Thus, it may be possible that CD1c$^+$ DCs from aged may be defective in antigen cross-presentation and may contribute to the reduced response to influenza observed in the elderly.

Nutrient sensing and electron transport pathways were also altered in DCs from aged individuals (Figure 4, Supplementary Table 3). Energy production is deficient while the expression of solute transporters is upregulated (Figure 4). Mitochondrial dysfunction is a hallmark of aging and is thought to be responsible for many of the age-related disorders (39). Defects in mitochondrial respiration are thought to enhance reactive oxygen species (ROS) production. The reduced energy generation also leads to a progressive decline in cellular and tissue function and/or increased susceptibility to apoptosis (40). Murine studies substantiate these observations as CD8$^+$ DCs from aged mice displayed profound signs of mitochondrial dysfunction. They exhibited reduced mitochondrial membrane potential, energy production and baseline oxidative phosphorylation while the proton leak and ROS production was increased (38). The upregulation of solute transporters particularly of glucose could be one of the ways to compensate for reduced energy generation by providing enhanced levels of the energy inducing substrates. Furthermore, it also indicates metabolic changes in DCs from aged subjects. Emerging studies have highlighted the role of glucose metabolism pathways, glycolysis and oxidative phosphorylation in regulating DC responses. Similar to tumor cells, activated DCs have been reported to generate ATP mainly by glycolytic degradation of glucose. The serine/threonine kinase mammalian target of rapamycin (mTOR) has emerged as a central regulator of anabolic processes in immune cells including DCs (41–44). mTOR promotes glycolytic response in activated DCs which supports antigen uptake and cytokine production. Amino acid starvation inhibits this pathway (43). mTORC1 is a signaling complex that senses the cellular status of amino acid nutrition (45). Since glucose and amino acids are the primary activators of mTORC1 signaling and expression of transporters for both are increased in DCs from aged individuals, it indicates an activated state of DCs from aged individuals. This is in keeping with previous studies where also an increase in activation of mDC was observed at the basal level. Decreased oxidative phosphorylation is also reported in DCs from aged mouse (38). Furthermore, increased mTOR activation is a hallmark of aging, and its inhibition via rapamycin has proven efficacious in increasing longevity and reducing inflammation in aging (46,47). Interestingly, rapamycin treatment of mouse bone marrow-derived DCs enhances their survival and the expression of co-stimulatory molecules making them more effective at inducing T cell responses (48). In contrast, mTOR activation has also been reported to prevent transcription of new MHCII to allow transport of already synthesized MHCII molecules to the surface (42). Similarly, Zn supplementation has also been reported to inhibit the up-regulation of MHCII and of co-stimulatory molecules in DCs (49). Decreased expression of antigen presenting molecules (Figures 2 and 3) could be due to enhanced expression of Zn, amino acid, and sugar transporters. In summary these changes suggest that function of CD1c$^+$ DCs may be changed substantially with age.

We also observed enhanced upregulation of chemokine receptor and SIP genes indicating an activated state of DCs in aged subjects. SIPR4 is expressed on DCs from mouse lungs while SIPR1 and SIPR3 expression is increased on DC maturation which facilitates DC migration (50). Kv channels genes were also upregulated and increased expression of Kv channels is associated with DC maturation (51,52). Moreover, Kv channels sustain Ca^{2+}influx through Ca^{2+} release-activated channels (CRAC) by maintaining the negative membrane potential and providing the necessary electrical driving force in DCs. Accordingly, Kv channel inhibitors impair Ca^{2+}-dependent cytokine production, maturation, and migration of murine DCs (53). Migration of monocyte-derived DCs in response to LPS has been reported to be defective in aged individuals (13) but there is a scarcity of information about migration of circulatory DC subsets. Interestingly, the expression of other molecules which indicate an activated state of DCs such as DC-LAMP is also upregulated in DCs from aged (54). Another class of genes which displayed significant upregulation in DCs from aged were the histones, small nucleolar RNA H/ACA box (SNORA) and small nucleolar RNA C/D/box (SNORD), and long non-coding RNA (lncRNA) genes. An example is provided in Supplementary Figure 3. The genes for Histone cluster 1 were increased with age (Supplementary Figure 3). Histone repressor, H3K9me3, H3K27me3, and activator H3K4me3 all belong to this cluster. Increased expression of histones has been associated with chromosome loss (55), increased DNA damage sensitivity and cytotoxicity (56). Furthermore excess histones can stick nonspecifically to the DNA and affect chromatin structure and gene expression (57). SNORA and SNORDs are noncoding RNAs which are involved in post-transcriptional modification of ribosomal RNA which are lead to changes in gene expression (58). Overexpression of SNORA and SNORDs has been observed in many cancers as well as COPD. LncRNA have also emerged as regulators of gene expression. They can induce post-transcriptional modification in gene expression and changes in their expression and function has been reported in many diseases (58). Altogether, these gene changes indicate that gene expression in DCs from aged individuals may be influenced by post-transcriptional modification of RNA.

In summary, we demonstrate substantial changes in gene expression profile of CD1c+ DC subset from aged subjects as compared to young subjects. DCs from aged subjects display significant upregulation of nutrient sensing and activation pathways coupled with downregulation of antigen-presenting and energy-generating genes. Further studies are required to determine whether these functions are conserved in CD1c+ DC in other tissues. CD1c+ DC in blood and lymphoid tissues are closely related to each other whereas CD1c+ DC in nonlymphoid tissue are heavily influenced by their microenvironment and may therefore function differently to their blood counterparts (11).

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of interest statement

None declared.

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