Quantitative Profiling of Differentially Produced Oxylipins in Human Adipose Derived Mesenchymal Stem Cells Under Proinflammatory Stimulation

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Research

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Abstract

Background: MSCs have been proven to have immune modulation and anti-inflammation capabilities, but the mechanisms are still under investigation. Recently, oxylipins have been identified to be involved in the immuno-regulation function of MSCs. However, the impact of proinflammatory stimulation on the profiles of MSCs derived oxylipins has never been studied.

Methods: In the present research, we employed a newly developed UPLC-MS/MS method to identify and quantify the oxylipin profile of ADSCs under proinflammatory stimulation (TNF-α and IFN-γ).

Results: As a result, among the analyzed 71 oxylipins, we detected and quantified 49 oxylipins derived from six major n-6 and n-3 polyunsaturated fatty acids (PUFAs) including arachidonic (AA), linoleic (LA), alpha-linolenic (ALA), dihomo-γ-linolenic acid (DGLA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids in all samples, while 4 oxylipins were detected only in a part of samples and 18 were not detected in all samples. Nine oxylipins were found to be significantly differentially produced in ADSCs after 24 hours of inflammatory stimulation, among which, several oxylipins were reported to be having anti-inflammation or proresolving functions and involved in lipid mediator switching.

Conclusions: The results reported here make a fundamental step towards a comprehensive characterization of MSCs derived oxylipins as potential modulators of inflammation and immunoreaction.

1. Introduction

Mesenchymal stromal/stem cells (MSCs) are multipotent progenitor cells that have the potency of differentiating into a variety of cell types, including osteoblasts, chondrocytes, adipocytes, skeletal myocytes, and cells of visceral mesoderm [1–3]. MSCs have been proven to have the capability of regulating immune responses. The immunosuppressive capacity of MSCs renders them promising candidates for treating diverse immune disorders. For example, *in vitro* expanded MSCs have already been applied in preclinical and clinical studies to treat diseases such as acute graft versus host disease (aGvHD), type I diabetes, multiple sclerosis, systemic lupus erythematosus and Crohn's disease [4]. Particularly, MSCs transplantation therapy has been found effective in patients with acute respiratory distress syndrome caused by COVID-19 virus infection, and the treatment is under intense clinical study.

However, the mechanisms by which MSCs regulate the immune response during various pathological processes are still under intense study. Recently, oxylipins, including lipoxins and resolvins, have been identified as essential regulators of the resolution phase of inflammation [5] and as important players in the immuno-regulation function of MSCs [6]. Oxylipins are bioactive lipids generated by the oxidation of polyunsaturated fatty acids (PUFAs) and are involved in inflammation, immunity, cell proliferation, apoptosis, tissue repair, and vascular functions, etc [7]. Because many of the oxylipins are isomers, it makes the identification and quantitation of dozens of oxylipins in a single biological sample a challenging task. In addition, most oxylipins are present at very low concentrations and are unstable in

biological samples, and some of these compounds can vary in concentration by more than several orders of magnitude. However, with the advancement in detection and quantification methods, we can now quantify dozens of oxylipins simultaneously with accurate nanomolar detection using an array of state-of-the-art mass spectrometry instruments.

It has been proven that the immuno-suppressive effect of MSCs is not constitutive. The vast majority of studies have demonstrated that MSCs can become highly immunosuppressive upon stimulation by inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-1α (IL-1α), interleukin-1β (IL-1β) or others, among which, the combination of IFN-γ plus TNF-α were most frequently used.

The aim of this paper is to examine the variation in the oxylipin profiles of MSCs under proinflammatory environment, induced by the presence of TNF-α and IFN-γ, using the most modern lipidomic approach. A highly sensitive ultraperformance liquid chromatography (UPLC) coupled to electrospray ionization (ESI) tandem mass spectrometry (MS/MS) method for bioactive oxylipin profiling was built previously to detect even the slightest alteration in human adipose derived mesenchymal stem cells (ADSCs) upon stimulation. As a consequence, we simultaneously profiled and quantified 71 targeted oxylipins in ADSCs with or without proinflammatory stimulation. The differentially produced oxylipins were in depth analyzed using bioinformatic tools for the first time to our knowledge. The result of this analysis may lay the foundation for the comprehensive understanding of the biofunction of MSCs derived oxylipins.

2. Materials And Methods

2.1 Chemicals and reagents

All oxylipins and deuterated internal standards were purchased from Cayman Chemical. HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). MilliQ water (Millipore, Bradford, USA) was used in all experiments. Acetic acid was purchased from Sigma–Aldrich. CNW Poly-Sery MAX SPE cartridges were from ANPEL Co. (Shanghai, PRC). The stock solutions of the standards were prepared at the concentration of 0.1 mg/mL in MeOH. All stock solutions were stored at -20 °C. The stock solutions were diluted with MeOH to working solutions before analysis.

2.2 Cell isolation and culture

Liposuction aspirates from subcutaneous adipose tissue samples were obtained from the waists of healthy donors (n = 3) undergoing elective plastic surgical procedures. It was done according to the protocol approved by the Ethics Committee at the Yongchuan Hospital of Chongqing Medical University (Chongqing, China), and all patients were provided informed consent. The obtained adipose tissue was washed twice with phosphate-buffered saline (PBS) and then digested with 0.25% type I collagenase (Sigma) under gentle shaking for 60 min at 37 °C. The digested tissue was filtered with a 200-mesh screen to remove the undigested tissue fragments. The suspension was neutralized with complete cell culture media and centrifuged at 400×g for 10 min. The supernatant was aspirated and the cell pellets

were washed three times with PBS. All acquired cells were cultured 24 hours at 37 °C in 5% CO2 in ADSC culture medium (F12 basic culture medium containing 10% FBS). Once adherent cells were observed, the medium was changed for the first time. The obtained cell population was passaged about 3 days until reaching 70–80% confluence, which was considered as passage 1. The ADSCs were cultured and expanded in culture medium, and passages 3–7 were generally utilized in the subsequent experiments.

2.3 Flow cytometric characterization of ADSCs

ADSCs were characterized by fluorescence activated cell sorting (FACS) analysis for surface antigens; cell surface expression of CD73(FITC), CD44(FITC), CD105(PE-Cy7), and CD90(FITC) and the absence of surface expression of CD34(APC) (these antibodies were purchased from eBioscience, USA). Flow cytometry was performed as described. A total of 2×10^6 cells were incubated with FcR blocking reagent and then stained with monoclonal antibodies; the labeled cells were subsequently analyzed by flow cytometry (FACS Calibur, Beckton Dickinson, Bedford, MA, USA).

2.4 Validation of the differentiation potential of ADSCs

ADSCs (3×10^3 cells/cm^2) were incubated until they attained 90% confluency. Adipogenesis was induced using a commercial kit from Stemcell Technologies and examined for the formation of oil globules by Oil Red O staining (Solarbio, China).

For osteoblast differentiation, monolayer cultures of ADSCs (3×10^3 cells/cm^2) were incubated in osteogenic differentiation media (Cyagen, China) for 21–25 days. Cultures were half fed every three days and assessed for matrix mineralization by Alizarin red staining (Solarbio, China).

For chondrogenic differentiation, ADSCs (3×10^3 cells/cm^2) were pelleted in round-bottom 96-well plates to form a pelleted micromass at the bottom of the well. Pellets were incubated in chondrogenic differentiation media (Stemcell Technologies, Canada). After 21 days induction, cell pellets were fixed in 4% polyoxymethylene and stained with Alcian Blue (Cyagen, China).

2.5 Inflammation stimulation and cell sample collection

Cells were seeded at a density of 15,000 cells/cm^2 and allowed to adhere for 24 hours. Then the supernatant was replaced with fresh culture medium supplemented with or without recombinant human IFN-γ (20 ng/ml, Prepotech) and TNF-α (20 ng/ml, Prepotech) [8]. After 48 hours of stimulation, the culture medium was discarded, and cells were washed twice with pre-cooled PBS. ADSCs were harvested and stored at -80 °C until further analysis.

2.6 Sample preparation and extraction

Cell samples were spiked with 200 μL of ice-cold methanol, vortexed for 5 minutes, and the proteins were precipitated at low temperature (-20 °C). Samples were then spiked with 20 μL of 1 μM internal standard mixture and vortex for 10 min, and further centrifuged at 5000 rpm for 10 min at 4 °C. Repeat the extraction once and combine the supernatants. The eicosanoids in supernatants were extracted using

Oasis SPE columns (MAX, 60 mg, Waters). Specifically, columns were first washed with 3 mL of MeOH and then equilibrated with 3 mL of H_2O. Homogenized samples were acidified with 0.1 M hydrochloric acid to give pH of 3.0. After loading the sample, the columns were washed with 10% MeOH to remove impurities, and the metabolites were then eluted with 1 mL of MeOH and stored at − 80 ◦ C to prevent metabolite degradation. Prior to analysis, the eluent was dried under vacuum and re-dissolved in 100μL of methanol/water (1:1, v/v) for UPLC/MS/MS analysis.

2.7 HPLC Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (Ultra Performance Liquid Chromatography, UPLC, ExionLC AD,https://sciex.com.cn/; MS, QTRAP® 6500 + System, https://sciex.com/). The analytical conditions were as follows, HPLC: column, Waters ACQUITY UPLC HSS T3 (100 mm×2.1 mm i.d.□1.8 μm); solvent system, water with 0.04% acetic acid (A), acetonitrile with 0.04% acetic acid (B); The gradient was 0 − 2.0 min from 0.1–30% B;2.0 − 4.0 min to 50% B; 4.0 − 5.5 min to 99% B, which was maintained for 1.5 min; and 7.0 − 8.0 min reduced to 0.1% B and maintained for 3.0 min. flow rate, 0.4 mL/min; temperature, 40 °C; injection volume: 10 μL.

2.8 ESI-MS/MS Conditions

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole linear ion trap mass spectrometer (QTRAP), QTRAP® 6500 + LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature, 550 ℃; ion spray voltage (IS)-4500 V; ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 50, 60, and 35 psi, respectively; the collision gas (CAD) was medium. Eicosanoids were analyzed using scheduled multiple reaction monitoring (MRM).

Mass spectrometer parameters including the declustering potential (DP) and collision energy (CE) E for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

2.9 Data processing and bioinformatics analysis

Data acquisitions were performed using Analyst 1.6.3 software (Sciex). Multiquant software (Sciex) was used to quantify all metabolites. Annotation was performed by using our in-house ESI-mass spectra database, as well as the KEGG(Kyoto Encyclopedia of Genes and Genomes) and the Human Metabolome Database (HMDB) public databases.

2.10 Statistical analysis

Statistical analysis was performed with GraphPad Prism 3.02 v Software (La Jolla, USA). Comparison was made using the Student’s t-test. Data were considered statistically significant when p-value was < 0.05. All analyses were performed in R software 3.6.2.

3. Results

3.1 Characterization of ADSCs

ADSCs were isolated and expanded from healthy human adipose tissue. ADSCs exhibited a fibroblast-like morphology (Fig. 1A). They maintained multipotency and differentiated into adipocytes, osteocytes, and chondrocytes (Fig. 1B) under in vitro culture conditions. In addition, they were uniformly negative for CD34, but positive for CD44, CD73, CD90, and CD105, in accordance with accepted phenotypic markers for ADSCs (Fig. 1C).

3.2 Oxylipins profiling and quantification in human ADSCs

The metware database (MWDB) of oxylipin was constructed based on the analysis of oxylipin standards. The qualitative and quantitative analysis of the acquired MS/MS data was carried out afterwards. The total ion chromatogram (TIC) of 71 oxylipin standards in the oxylipin metabolome was illustrated in Fig. 2. Within ten minutes, all 71 compounds could be separated and measured accurately. Representative chromatograms depicting the LC/ESI-MS/MS analysis of a standard mixture of all measured compounds are shown in Fig. 3. The repeatability of the extraction and detection of oxylipins is judged by the overlapping display of total ions current (TIC) of different quality control samples. As shown in Fig. 4, the results showed that the peak intensity and retention time were highly consistent, which indicated that the signal stability was good when the same sample was detected at different times.

Quantification of 71 target lipid metabolites was achieved using stable isotope labeled internal standards and commercially available primary standards; all targets are quantified using a calibration curve prepared for primary standards of known concentrations, which are also mixed with internal standards (see supplementary table S1). Quantitation of oxylipin production was calculated as the peak area ratio of the target analyte to the corresponding internal standard. The limit of detection (LOD) was calculated using a signal-to-noise ratio (S/N) of 3, while the limit of quantification (LOQ) was determined using a signal-to-noise ratio of 10.

Applying the oxylipin profiling platform to human ADSCs samples, we were able to detect and quantify 49 oxylipins derived from six different categories of PUFAs in all cell samples with the minimal LOQ of 0.01 nmol/L, while 4 oxylipins were not detected except in one or two samples, 18 compounds were not detected in all samples (see supplementary table S2). The majority of oxylipins quantified in ADSCs are assigned to AA, followed by metabolites derived from EPA, DHA, and LA. All these metabolites are biosynthesized via the three enzymatic COX, LOX, and CYP450 pathways, especially the COX pathway.

As the most prominent metabolites of the COX pathway, prostaglandins PGE2 and PGJ2 as well as thromboxane B2, derived from AA, were detected. Furthermore, prostaglandin E1 and prostaglandin D1 were detected, which were generated from DGLA as a precursor, while no EPA derived 3 series prostaglandins were detected. It’s not surprising that none of the resolvins (RvD1, RvD2, RvD3, RvD5,

RvE1) were detected in any sample, because the resolvins were mainly synthesized by macrophages and neutrophils referring to previous reports.

3.3 Data analysis and differentially produced oxylipins

Based on the qualitative and quantitative analysis results, the variations in the oxylipin profiles between stimulated and none-stimulated ADSCs were compared. Fold change ≥ 2 or fold change ≤ 0.5, combining VIP ≥ 1, were considered significantly changed between the two groups. As a result, according to this criterion, 9 oxylipins were found to be overexpressed while none underexpress in ADSCs under the activation of proinflammatory cytokines (Fig. 5). PGE2 showed the most significant variation (~ 5.88-fold overproduction). PGJ2 (~ 4.75-fold overproduction) and PGE1 (~ 3.91-fold overproduction) levels also increased considerably under stimulation compared with the control cells.

Hierarchy clustering analysis (HCA) was subsequently applied to evaluate the similarity of oxylipin patterns between naïve and pro-inflammatory stimulated ADSCs. The HCA-heatmap was conducted using R software (https://www.r-project.org/), and the result was shown in Fig. 6 (A). However, there were variations in oxylipin production from different ADSC donors. Comparative analysis results of 9 oxylipins in the two groups were illustrated in the following violin plot, which combines a box plot and density map, demonstrating the data distribution and probability density, Fig. 6 (B).

3.4 Bioinformatics analysis of oxylipins identified in human ADSCs

To explore the metabolic fluctuations in ADSCs upon stimulation, pathway and functional analysis was carried out based on the integration of the oxylipin data set. Pathway associations determined using KEGG Metabolic Mapper function using high confidence metabolites with peak intensity significantly above background, n = 3. Data was exported as a table and several graphs. Typical pathways with an adjusted $P < 0.05$ and pathways with two or more identified target molecules were reported as graphs.

Bioinformatic pathway analysis databases are often curated from different sources. We used HMDB to annotate the differentially expressed oxylipins, which allows for the simultaneous investigation of several databases, including KEGG, Metlin, and Biocyc, besides the endogenous database. The bioinformatics findings from one of the analyzed databases (SMPDB) are listed in supplementary table S3, and a representative metabolic pathway is depicted in Fig. 7.

4. Discussion And Conclusions

Due to their capacity of multi-lineage differentiation and immunomodulation, MSCs have got great interest in various cell-based therapies, including the treatment or prevention of acute graft versus host disease, autoimmune diseases, and solid organ transplantation rejection. Multiple mechanisms underlying the MSC immunosuppressive effect have been elucidated, however, understanding of the

underlying mechanisms remains far incomplete. As far as we know, to date, these MSC properties are largely due to the paracrine effects from the secretion of biologic factors.

Among the secreted factors, prostaglandins (PGs), a category of oxylipins, have been long associated with inflammation and targeted by cyclooxygenase (COX) inhibitors to treat inflammatory diseases. PGs are traditionally considered proinflammatory oxylipins, but a previous study [9] showed that MSC, but not fibroblasts exposed to carbon monoxide, with docosahexaenoic acid substrate, produced proresolving oxylipins, particularly D-series resolvins, contributing to attenuating systemic inflammation and sepsis.

It is not the first time that oxylipins were reported to be involved in MSC's immuno-modulatory function. In another report, experiments provide evidence that human MSCs can promote the resolution of acute lung injury in mice, in part through the pro-resolving oxylipin, LXA4, and LXA4 itself could be considered as a therapeutic for acute respiratory distress syndrome (ARDS), mediating part of the potential therapeutic effects of MSCs [10]. Furthermore, one of these MSC derived prostaglandins, prostaglandin E2 (PGE2), has been recognized as having anti-inflammatory and immunomodulatory effects. PGE2 initiates lipid mediator class switching, which results in a decrease in 5-lipoxygenase (LOX)-derived pro-inflammatory lipid mediators and an increase in 15-LOX-derived pro-resolving oxylipins [11].

It has been indicated in a previous review that inflammation and lipid signaling are intertwined modulators of homeostasis and immunity. Oxylipins and other lipid mediators' function to regulate inflammatory responses both positively and negatively. Conversely, inflammatory signaling can also significantly affect the lipid oxidation process in MSCs. Furthermore, injurious stimuli (i.e., infection and inflammation) can induce the acute-phase response and lead to multiple alterations in the metabolism of lipids. Thus, it is critical to perform a detailed characterization of the altered oxylipin metabolism depending on inflammation in MSCs. And the findings may lay a great foundation for MSCs based therapies and further determine the ways to improve the therapeutic potential of MSCs.

A more detailed investigation of the downstream metabolic products of PGs and other polyunsaturated fatty acids (PUFAs) in ADSC is needed. To our knowledge, ADSCs derived oxylipins have never been comprehensively analyzed. Oxylipins are biosynthesized by enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids (PUFAs), which form eicosanoids from the metabolism of 20-carbon arachidonic acid (AA) and eicosapentaenoic acid (EPA), 22-carbon docosanoids from docosahexaenoic acid (DHA), and 18-carbon oxidized metabolites of linoleic acid (LA) and linolenic acid (ALA). After decades of research, numerous biologic functions have been linked to them and many others are still being elucidated. Quantification of eicosanoids and oxylipins derived from other PUFAs in biological samples is crucial for a better understanding of the biology of these lipid mediators.

However, the analytical determination of these metabolites is challenging. Usually, the endogenous concentrations of these oxylipins are very low and they are instable in biological samples. The structural similarity between members of the oxylipin metabolome, particularly the isomers, adds difficulty in chromatographic separation of these compounds. The ideal analytical method should be able to simultaneously profile and determine all relevant species in a single analytical assay, because these

compounds are produced within the same cascade and they all are part of a complex regulatory network. Therefore, a highly sensitive and selective analytical method is required for the comprehensive study of this class of lipids.

We utilized a sensitive and selective ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the simultaneous profiling of 71 targeted oxylipins in ADSCs with or without proinflammatory stimulation. These quantified oxylipins are derived from six major n-6 and n-3 polyunsaturated fatty acids (PUFAs) which serve as oxylipin precursors, including arachidonic (AA), linoleic (LA), alpha-linolenic (ALA), (DGLA) eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids. The targeted oxylipin panel provides a broad coverage of lipid mediators and pathway markers generated from cyclooxygenases, lipoxygenases, cytochrome P450 epoxygenases/hydroxylases, and non-enzymatic oxidation pathways. The limits of quantification (LOQ) and detection (LOD) were set at a signal-to-noise ratio (S/N) of 10 and 3, respectively.

Detection and quantification of critical oxylipins in inflammation are essential for immunological disease surveillance and therapeutic target discovery. As we know, MSCs express cyclooxygenase 1 and 2 and produce prostaglandin E2 (PGE2) constitutively. In accordance with our expectations, PGE2 was found highly produced in both stimulated or unstimulated cell samples (~5.88-fold overexpressed in stimulated cells), probably serving as the major precursor and switcher of many downstream oxylipins [11].

An eicosapentaenoic acid (EPA)-derived eicosanoid, 15-HEPE, which associates with anti-inflammatory properties, was found apparently upproduced (~2.2-fold) in inflammatory-stimulated ADSCs. Earlier studies have shown that 15-HEPE and 15-hydroxyeicosatetraenoic acid (15-HETE) inhibit 5-LOX activity and proinflammatory prostaglandin synthetase [12]. The result in a decrease in 5-lipoxygenase (LOX)-derived proinflammatory lipid mediators and an increase in 15-LOX-derived proresolving oxylipins, which resembles the initiation of resolution of inflammation. Additionally, it was reported that 15-HETE and 15-HEPE are converted to lipoxins via 5-hydroperoxy, a 15-hydroxy acid derivative [13]. These lipid mediators, actively biosynthesized in the resolution phase of acute inflammation, control the duration and magnitude of inflammation. In a recent study, Kento Sawane and college identified 15-HEPE as a novel EPA-derived anti-inflammatory and/or proresolution lipid mediator in a PPARγ-dependent manner by 15-lipoxygenase activity in eosinophils [14]. Targeting the transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ), an important inflammation regulator, may serve as promising therapeutics to treat inflammatory disorders. Interestingly, a switch towards 15-lipoxygenase products, such as the pro-resolving lipid precursors 15-HEPE and 17-HDHA was observed after 24 h inflammatory stimulation in monocyte-derived macrophages.

Meanwhile, the levels of 7-HDHA, the pathway marker for D-series resolvins, were significantly higher in ADSCs subjects after stimulation. However, none of the D-series resolvins (RvD1, RvD5, RvD2, RvD3) were detected in all cell groups. Hence, we speculated that 7-HDHA might be the substrate for transcellular biosynthesis of D-series resolvins in other pro-solving immune cells such as neutrophils or macrophages, which needs further investigation.

In conclusion, we introduced a targeted UPLC- MS/MS lipid mediator metabolomics strategy for the detection, identification, and quantification of 71 oxylipins derived from LA (n=6), AA (n=31), ALA (n=3), EPA (n=15), DGLA (n=5), DHA (n=8), and others (n=3) in MSCs under pro-inflammatory stimulation.

The activation with proinflammatory cytokines of ADSCs induced the significant overexpression of nine oxylipins and the underexpression of none oxylipin, which induces profound changes in the downstream oxylipin functioning in the resolution of inflammation. These data indicate that MSCs might contribute to the resolution of inflammation in time by the production of proresolving lipids after an initial inflammatory stimulus. The results reported here make the first step towards a comprehensive characterization of MSCs derived oxylipins as potentially modulators of inflammation and immune.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Ethics Approval and Consent to Participate

The research was approved by the Ethics Committee at the Yongchuan Hospital of Chongqing Medical University (Chongqing, China), and all patients were provided informed consent.

Consent for publication

Not applicable.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Dan Wang: Validation, Formal analysis, Visualization, Software.

Zhengze Shen: Conceptualization, Methodology, Investigation, Writing - Original Draft.

Chaoqun Yu: Resources, Validation, Visualization.

Lifang Cheng: Validation, Formal analysis.

Daorong Chen: Resources, Writing - Review & Editing, Supervision.

Yuan Zhang: Resources, Writing - Review & Editing, Supervision,

Data Curation.

Acknowledgements

Not applicable.

Abbreviations

Mesenchymal stromal/stem cells (MSCs); acute graft versus host disease (aGvHD); polyunsaturated fatty acids (PUFAs); ultraperformance liquid chromatography (UPLC); adipose derived mesenchymal stem cells (ADSCs)

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Figures

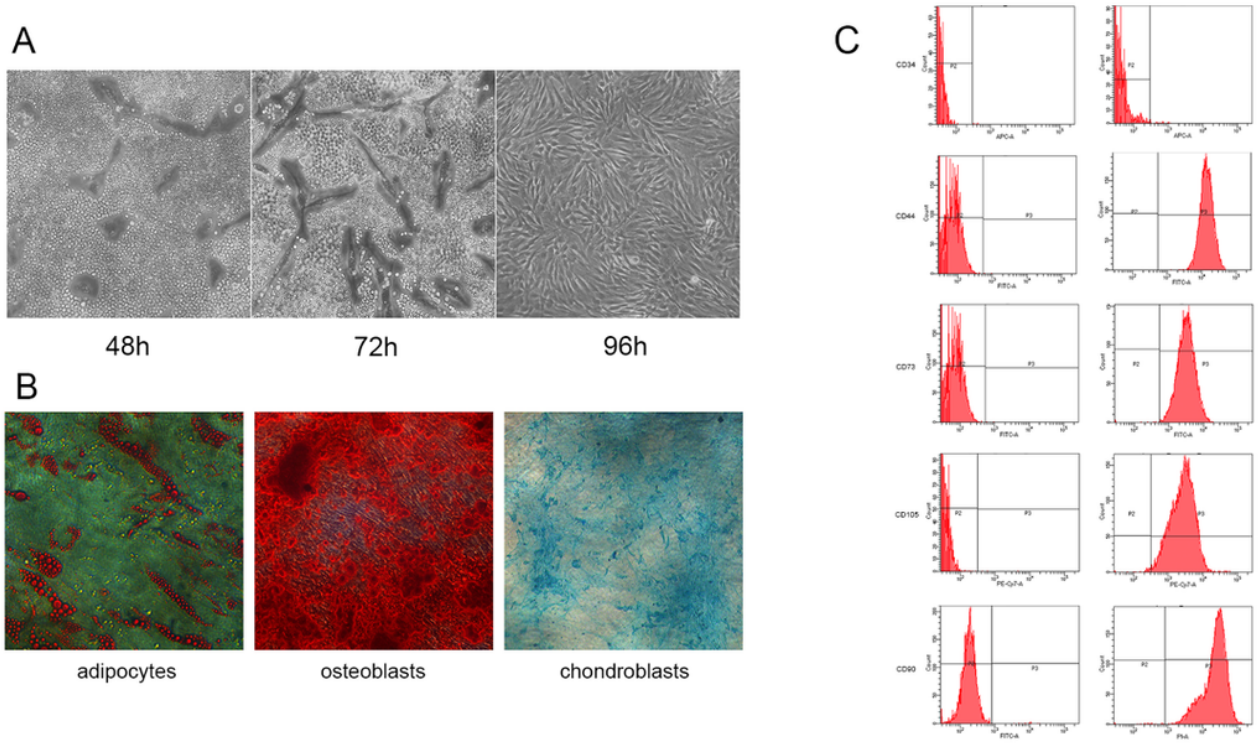

Figure 1

(A) Morphology of ADSCs at different times after isolation; (B) Differentiation into adipocytes, osteocytes, and chondrocytes. (C) The phenotype of ADSCs was analyzed by flow cytometry. ADSCs were positive for CD44, CD73, CD90 and CD105, and negative for CD34.

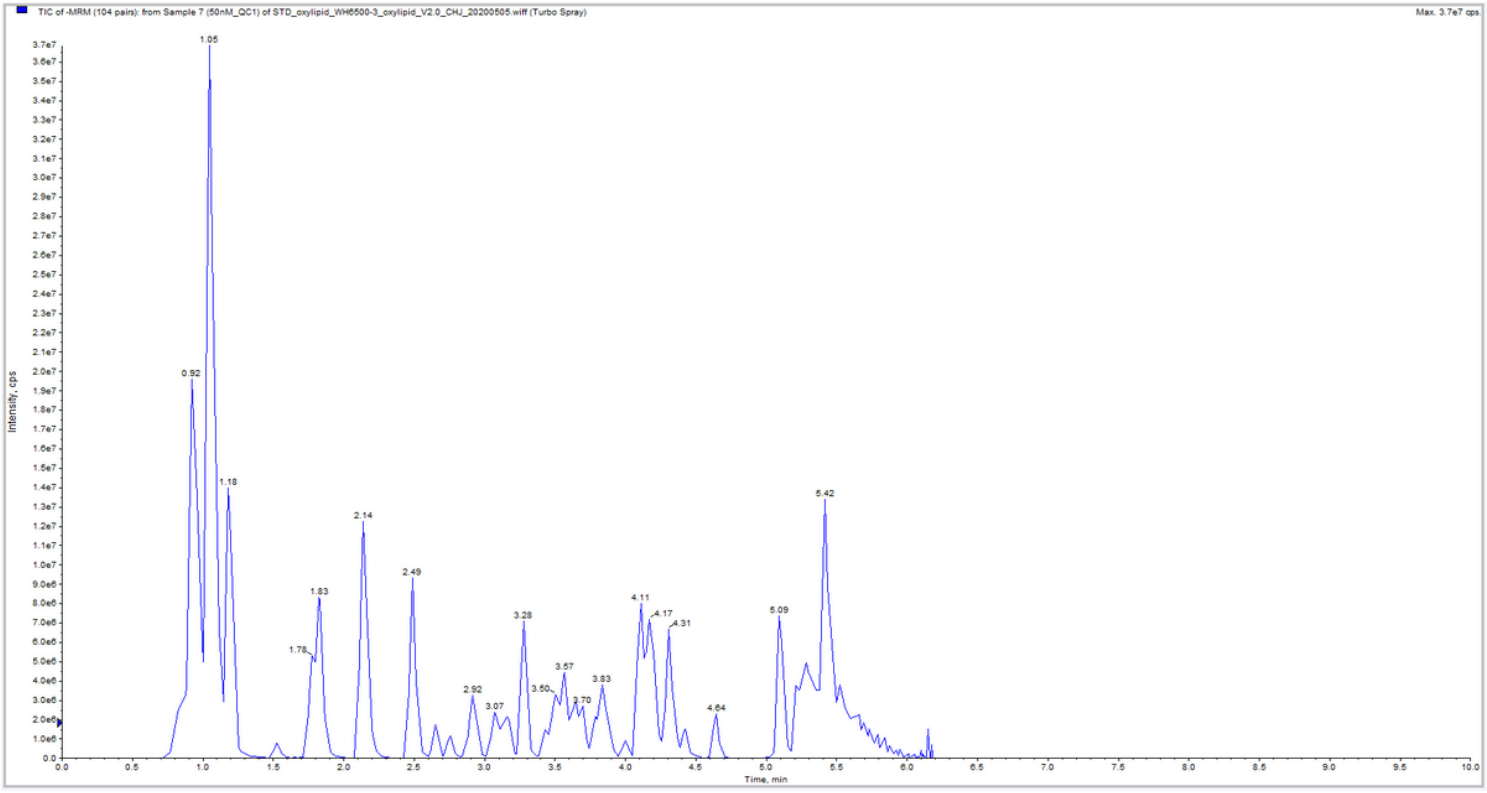

Figure 2

Total Ion Chromatogram (TIC) of 71 oxylipins standards in the oxylipin metabolome. In ten minutes, all 71 compounds could be separated and measured accurately.

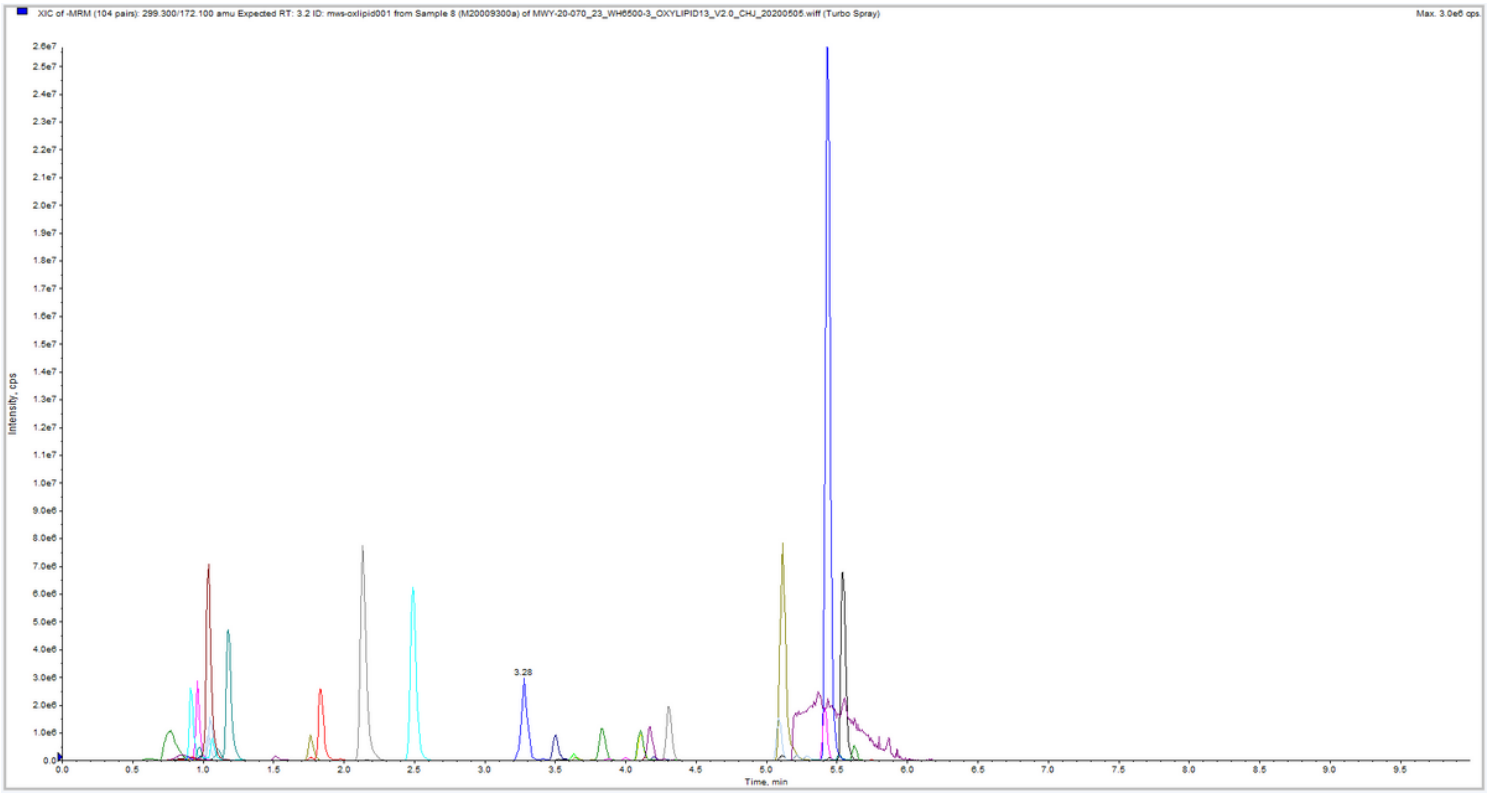

Figure 3

MRM chromatograms of each compound analyzed in a standard solution mixture. Each chromatogram peak of different color represents one metabolite.

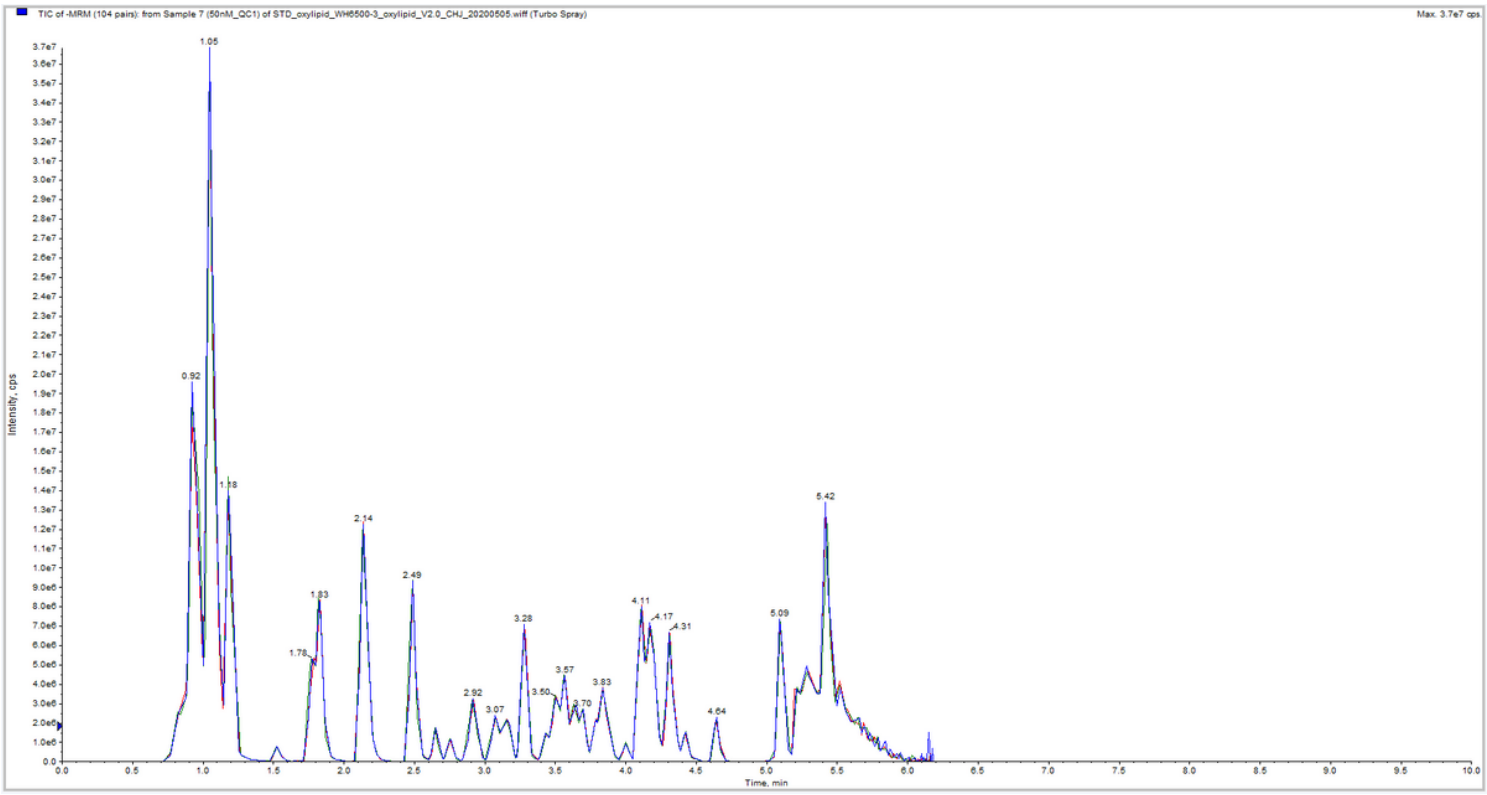

Figure 4

The repeatability of the extraction and detection of oxylipins is judged by the overlapping display of total ions current (TIC) of different quality control samples.

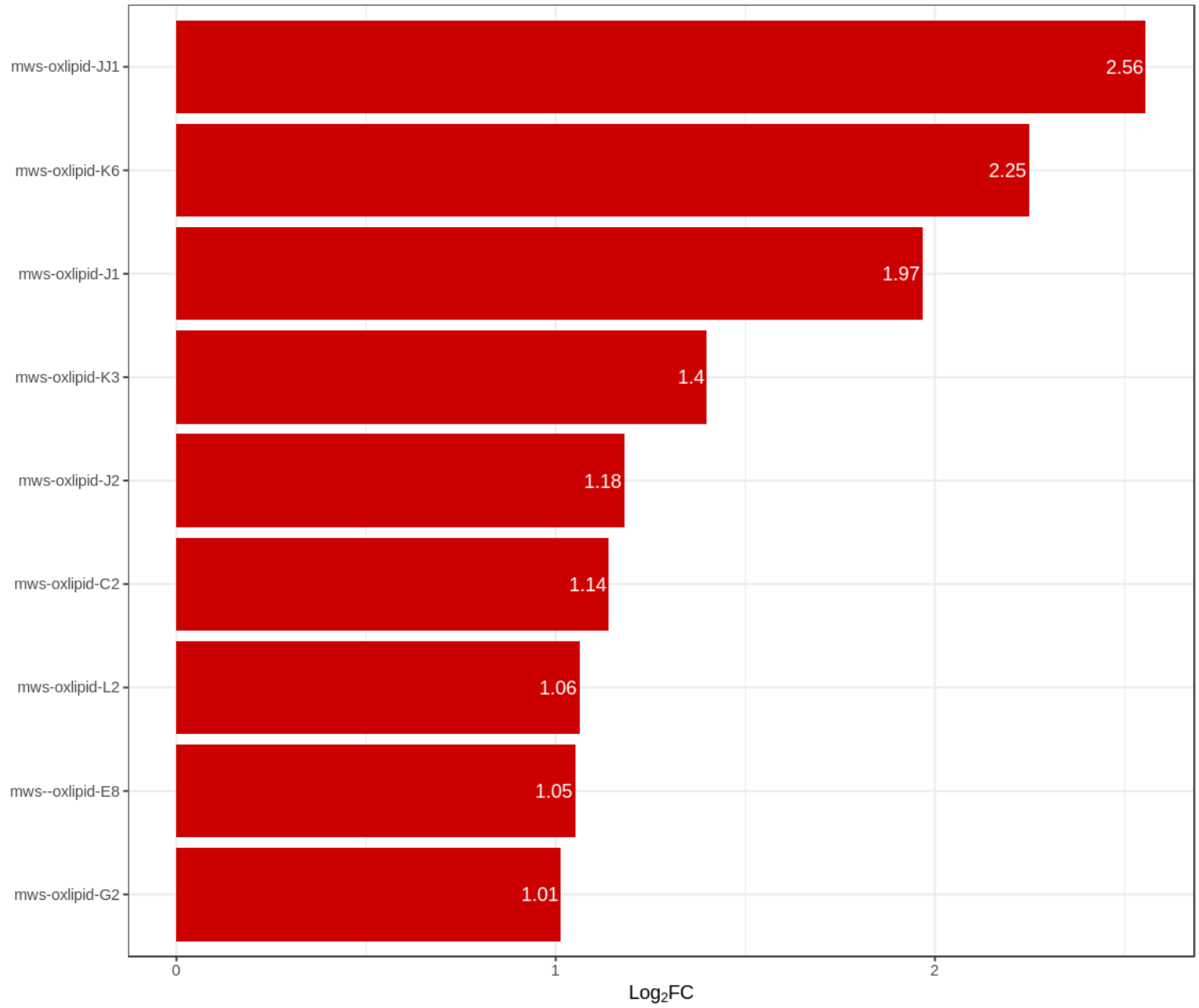

Figure 5

The differentially expressed oxylipins. The expression levels are present in log2 mean of relative oxylipin expression. The relative fold change is present in the log2 value between the two groups with the first group as the reference group. (mws-oxlipid-JJ1 refers to PGE2, mws-oxlipid-K6 refers to PGJ2, mws-oxlipid-J1 refers to PGE1, mws-oxlipid-K3 refers to PGF2α, mws-oxlipid-J2 refers toPGD1, mws-oxlipid-C2 refers to (±)15-HEPE, mws-oxlipid-L2 refers to TXB2, mws-oxlipid-E8 refers to (±)11-HETE, mws-oxlipid-G2 refers to (±)7-HDHA)

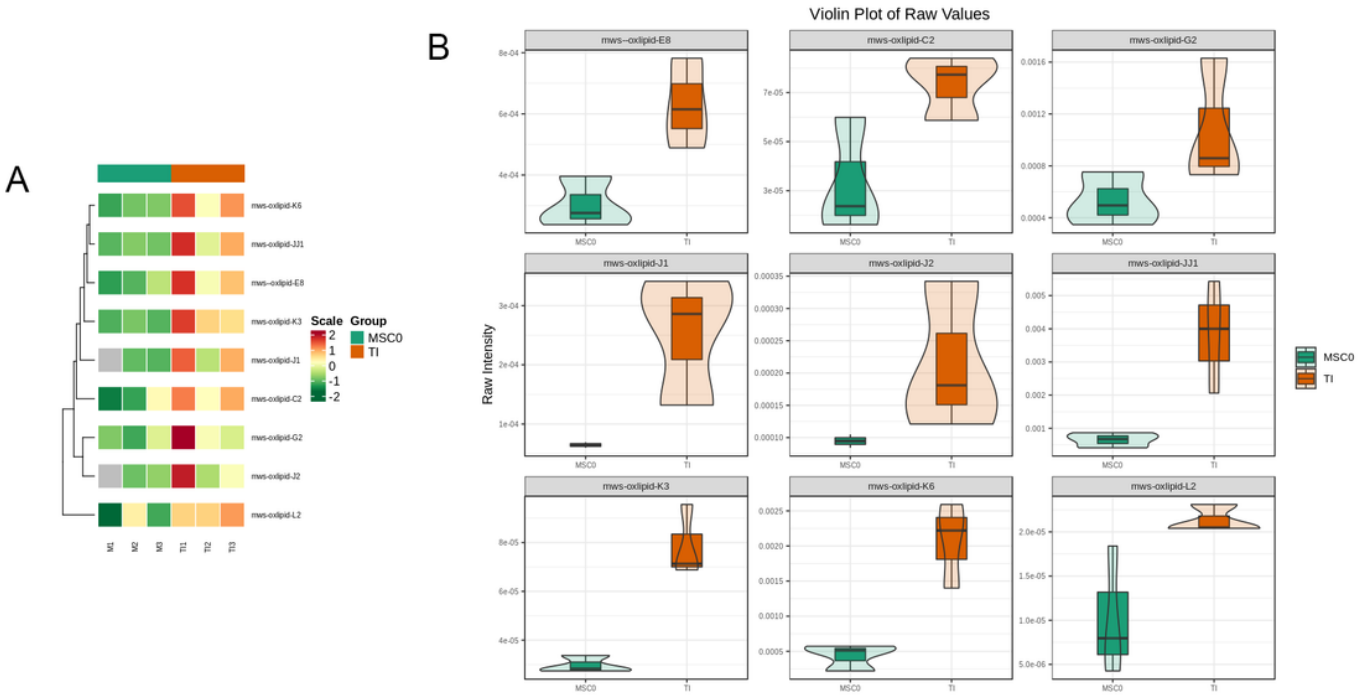

Figure 6

(A). Heat maps showed the variation of differentially produced oxylipins in ADSCs (MSC0) and pro-inflammatory stimulated ADSCs (TI). Colors represent increased (red) or decreased (green) abundance. (B). The violin plot of differentially produced oxylipins.

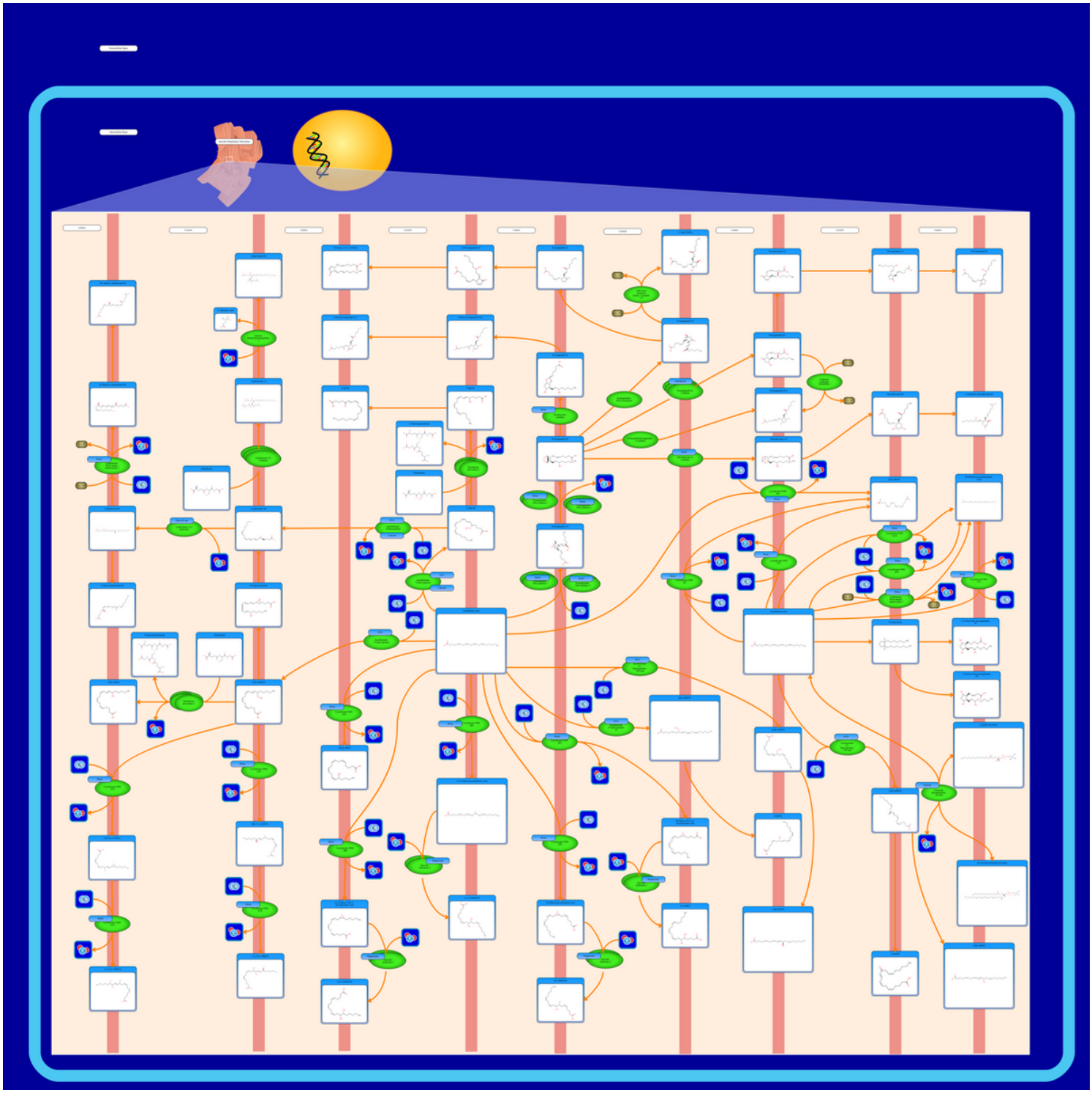

Figure 7

A representative metabolic pathway (AA metabolic pathway) is depicted in the bioinformatics findings from SMPDB databases.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS11009.pdf
- TableS21009.pdf
- TableS31009.pdf