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Data in Brief

Lysophosphatidic acid (LPA) 18:1 transcriptional regulation of primary human gingival fibroblasts

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ABSTRACT

The pleiotropic, bioactive lipid lysophosphatidic acid [(LPA), 1-acyl-sn-glycerol-3-phosphate] exerts critical regulatory actions in physiology and pathophysiology in many systems. It is present in normal bodily fluids, and is elevated in pathology (1). In vivo, "LPA" exists as distinct molecular species, each having a single fatty acid of varying chain length and degree of unsaturation covalently attached to the glycerol backbone via an acyl, alkyl, or alkenyl link. These species differ in affinities for the individual LPA receptors [(LPARs), LPA1-6] and coupling to G proteins (2). However, LPA 18:1 has been and continues to be the most commonly utilized species in reported studies. The actions of "LPA" remain poorly defined in oral biology and pathophysiology. Our laboratory has addressed this knowledge gap by studying in vitro the actions of the major human salivary LPA species [18:1, 18:0, and 16:0 (3)] in human oral cells (4–7). This includes gingival fibroblasts (GF), which our flow cytometry data from multiple donors found that they express LPA1-5 (6). We have also reported that these species are ten-fold elevated to pharmacologic levels in the saliva and gingival crevicular fluid obtained from patients with moderate–severe periodontitis (8). As the potential of LPA to regulate transcriptional activity had not been examined in the oral system, this study used whole human genome microarray analysis to test the hypothesis that LPA 18:1-treated human GF would show significant changes in gene transcripts relevant to their biology, wound-healing, and inflammatory responses. LPA 18:1 was found to significantly regulate a large, complex set of genes critical to GF biology in these categories and to periodontal disease. The raw data has been deposited at NCBI's GEO database as record GSE57496.

Specifications	
Organism/cell line/tissue	*Homo sapiens*/primary fibroblasts/gingiva
Sex	Male and female mix [3 donors/pool (pools = A, B, C)]
Sequencer or array type	Agilent-014850 Whole Human Genome Microarray 4x44K G4112F
Data format	Raw
Experimental factors	Normal vehicle-treated vs. LPA 18:1-treated (2 h, 8 h)
Experimental features	Transcriptional profiling of GF, comparing control GF with GF treated with LPA 18:1 for 2 h or 8 h. The goal of this study was to determine the effects of LPA 18:1 on GF global gene expression.
Consent	Open source; please cite study and URL in derivative works
Sample source location	Creighton University Dental School clinic patients, Omaha, NE, USA

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Direct link to deposited data

The data has been deposited with NCBI's GEO database as record GSE57496, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57496.

Experimental design, materials and methods

Oral fibroblast isolation and culture

Third molar extraction is routinely performed at Creighton University Dental Clinic. The gingival tissue used was attached gingiva obtained from healthy, young (mean age: 25–35 years; five males, four females) non-smoking patients of Caucasian descent. They were not taking any medications and had no evidence of periodontal disease. The study conformed to the Declaration of Helsinki guidelines and was approved by the Creighton University Institutional Review Board. Informed written consent was obtained from all donors. GF were isolated by rinsing the gingival tissue six times [in Hank's balanced salt solution (HBSS), Invitrogen, Grand Island, NY, USA], in

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order to diminish microbial contaminants and then finely mincing with scalpels inside a 35 mm dish (Falcon, Fisher Scientific, Waltham, MA, USA) containing 2 ml of complete media [Dulbecco's modified Eagle medium (DMEM, Invitrogen), with 10% defined fetal bovine serum (Hyclone, Logan, UT, USA) and 100 μg/ml Primocin (Invivogen, San Diego, CA, USA)]. The GF were allowed to explant from the minced tissue (mean time ~14 days) in complete media with changes every three days.

Of note, the GF isolates we have used to study mitogenesis, growth, chemotaxis, in vitro wound-healing, and elevation of intracellular calcium (4–7) were cultured from young, healthy male and female patients in the same age range as the donors for this study. Statistical analyses showed no detectable response differences to LPA 18:1 treatment between isolates.

LPA treatment

In order to preserve cellular responses closest to in vivo, pass 2 GF were used for mRNA isolation. The GF were seeded at 1×10^4 cells/2 ml in 35 mm dishes, and incubated in a 5% CO_2, 37 °C cell culture incubator for two days until approximately 90% confluent. The cells were washed four times with HBSS and then serum-starved for 24 h in serum-free DMEM with Primocin. This step was done to make sure that any LPA-mediated effects from the serum the cells were cultured in would be significantly diminished or absent. Human GF can be completely deprived of serum for this period of time (unpublished observations from our previous studies) without triggering apoptosis.

LPA (18:1) (Sigma-Aldrich, St. Louis, MO, USA) stock was prepared at 1×10^{-2} M in 0.25% de-lipidated (fraction V) bovine serum albumin (BSA) (Sigma-Aldrich) in serum-free DMEM. This is one of the major unsaturated LPA species (1,2) that is present in normal human saliva (3,8). Each donor's cells were individually treated for 2 h or 8 h with 2 ml 1×10^{-5} M LPA in serum-free DMEM. Controls were treated with an equal volume of 0.25% fraction V BSA in serum-free DMEM.

RNA isolation

The cells from each donor were individually trypsinized, washed once with ice-cold phosphate-buffered saline (PBS), held on wet ice, and counted. The cells from the nine donors were then grouped into 3 pools of 3000 cells in total, each containing 1000 cells/donor [pool A = donors 1–3 (2 M, 1 F), pool B = donors 4–6 (1 M, 2 F), and pool C = donors 7–9 (2 M, 1 F)]. Miltenyi's Biotec's (San Diego, CA, USA) proprietary SuperAmp™ buffer was used to directly lyse the cell pools using their protocol for cells (http://www.miltenyibiotec.com/~/media/Images/Products/Import/0006000/IM0006042.ashx). The lysates were quick-frozen on dry ice and shipped to the company for microarray analysis.

Microarray analysis

As we avoided larger-scale expansion of the pass 2 GF isolates beyond 35 mm dishes, the SuperAmp™ technology was then utilized at Miltenyi Biotec. Their proprietary amplification method is based on a global PCR protocol using cDNA derived from mRNA. Magnetic bead (MACS®) technology was used to isolate the mRNA. The average length of the highly amplified cDNA products ranged between 200–1000 bp. A ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to quantify the cDNA. The integrity of the cDNA was checked via the Agilent 2100 Bioanalyzer platform (Agilent Technologies, Santa Clara, CA, USA). The results of the Bioanalyzer run are visualized (Fig. 1) using the Agilent 2100 Bioanalyzer expert software. The gel image (A) and electropherogram (B) of the amplified cDNA samples are shown.

LPA-induced gene transcription for each treatment pool and controls was assessed by the company. cDNAs (250 ng of each sample) were used as template for Cy3 and Cy5 labeling, which was performed

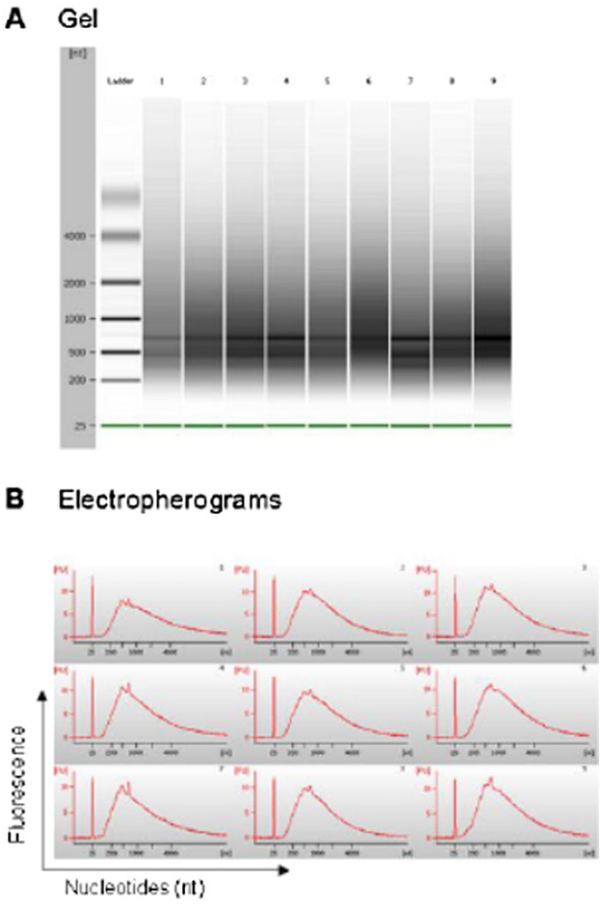

Fig. 1. Gel image (A) and electropherogram (B) of amplified cDNA samples. The first lane shows the reference DNA molecular weight ladder (in nucleotides, nt). The lowest migrating, green band is an internal standard. Scaling of the y-axis is done automatically, relative to the strongest signal within a single run.

according to Miltenyi's proprietary protocol. The corresponding Cy3- and Cy5-labeled cDNAs were combined and hybridized overnight (17 h, 65 °C) to an Agilent Whole Human Genome Oligo Microarrays 4 × 44 K using Agilent's recommended hybridization chamber and oven. Control samples were labeled with Cy3 and experimental samples were labeled with Cy5.

In the final steps, the microarrays were washed once for 1 min at room temperature with 6× SSPE buffer containing 0.005% N-lauroylsarcosine, followed by a second 1 min wash with pre-heated (37 °C) 0.06× SSPE buffer containing 0.005% N-lauroylsarcosine. The last washing step (30 s) was performed with acetonitrile.

Image and data analysis

The microarray image files were read out and processed using Agilent Feature Extraction Software (FES), which reads feature intensities and ratios (including background subtraction and normalization), rejects outliers and calculates statistical confidences (p-values). The output data of the Agilent Feature Extraction software includes gene lists with the complete raw data sets, referred to as single-experiment raw data list; the complete descriptions can be found in the Agilent G2567AA Feature.

Extraction software v.9.1 reference guide

For determination of differential gene expression FES-derived output data files, Miltenyi further analyzed the data using the Rosetta Resolver® gene expression data analysis system (Rosetta Biosoftware, www.rosettabio.com). This software's analytical range includes the

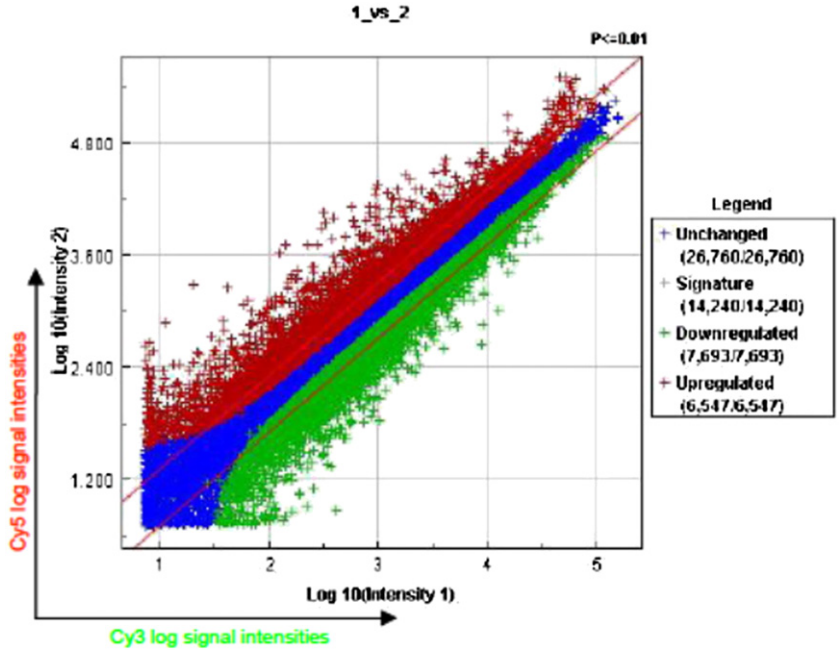

Fig. 2. An example the data. One array experiment (no. 1) is shown. This is a scatter plot of the signal intensities of all spots. The signal intensities of each feature are represented by a dot and shown in double logarithmic scale. X-axis: Cy3-log signal intensity; y-axis: Cy5-log signal intensity. Diagonal red lines define the areas of 2-fold differential signal intensities. Blue cross: unchanged genes. Red cross: significantly up-regulated genes (p-value < 0.01). Green cross: significantly down-regulated genes (p-value < 0.01). Gray cross in legend: summary of significantly up- and down-regulated signatures.

capacity to visualize the data analysis results as a double-log scatter plot. A double-log scatter plot for experiment number one is shown in Fig. 2 as an example. Rosetta Resolver® analysis results in a level of significance of $p \leq 0.01$. Thus, genes from the microarray experiments which showed $\geq$, or $\leq$ 2-fold differences between the signal from the LPA-treated samples and their untreated controls were designated as significantly changed.

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