Data in Brief

MicroRNA expression profile of bone marrow mesenchymal stem cell-derived neural progenitor by microarray under the influence of EGF, bFGF and IGF-1

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A R T I C L E   I N F O

A B S T R A C T

Recently there has been growing interest in the differentiation of mesenchymal stem cells (MSCs) into neural lineages. Research suggests that MSCs can be differentiated into neural progenitor-like cells (NPCs) under the specific influence of paracrine factors particularly epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Our recent research has found that the addition of insulin-like growth factor 1 (IGF-1) with the combination of the EGF and bFGF could significantly improve the growth and survivability of MSC-derived NPCs. To unravel the molecular mechanism of the improved differentiation we compared the microRNA expression profiles of the differentiation under various combinations of growth factors. MSCs were differentiated into neural lineage in 3 groups; Group A (EGF + bFGF), Group B (EGF + bFGF + IGF-1), and Group C (without growth factor). Regulated microRNAs during the early differentiation were identified by detailed microRNA profiling using Affymetrix GeneChip version 2.0 at three time intervals (day 1, day 3 and day 5). The data were deposited in the Gene Expression Omnibus, series GSE60060.
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Specifications	
Organism/cell line/tissue	*Rattus norvegicus*/Sprague Dawley rats' bone marrow-derived mesenchymal stem cells
Sex	Male
Sequencer or array type	Affymetrix GeneChip miRNA 2.0 arrays
Data format	Raw data: CEL files, Normalized data: CHP, Processed: SOFT, MINiML, TXT
Experimental factors	Group A (EGF + bFGF) vs. Group B (EGF + bFGF + IGF-1) vs. Group C (without growth factor)
Experimental features	MicroRNA microarray expression profiling to identify microRNAs that are regulated under the effects of growth factor-conditioned microenvironment.

(continued)

Specifications	
Consent	USM/Animal Ethics Approval/(59) (196)
Sample source location	Animal Research and Service Centre of Universiti Sains Malaysia (USM)

1. Direct link to deposited data

Deposited data are available here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE60060.

2. Experimental design, materials and methods

2.1. Experimental design

Mesenchymal stem cells (MSCs) isolated from rats' bone marrow tissue were differentiated into neural progenitor-like cells (NPCs) under different three combinations of growth factors: Group A —

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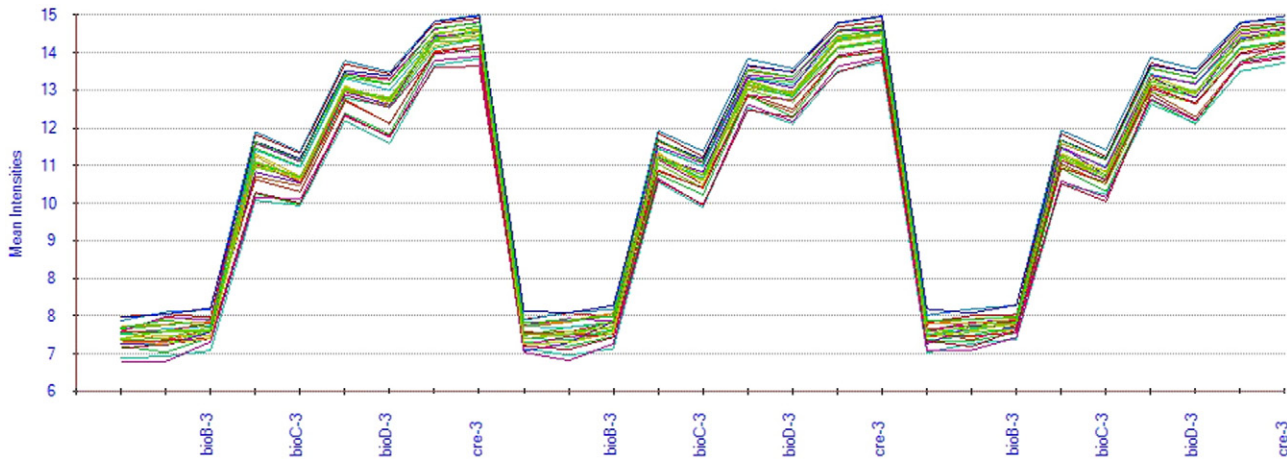
Fig. 1. Hybridization spike-in control signal commensurates with concentration: bioB-3 < bioC-3 < bioD-3 < creX-3, indicating good quality hybridization. Each line corresponds to an array.

EGF + bFGF, Group B — EGF + bFGF + IGF-1 and Group C without any growth factor that serves as negative control. MicroRNA expression profiles of early neurogenesis (day 1, day 3 and day 5) were analyzed using Affymetrix miRNA GeneChip 2.0. The raw data was deposited in the NCBI Gene Expression Omnibus, series GSE60060. Metadata for this research are available in the data supplement of Huat et al. [1].

2.2. Animal ethics clearance

All procedures involving animal were reviewed and received approval from the board of Animal Ethics Committee of Universiti Sains Malaysia (Document No.: USM/Animal Ethics Approval/(59) (196)).

2.3. Cell culture

Primary culture of MSCs was isolated from Sprague Dawley rats' bone marrow tissue by their selective adherence to plastic surface as described previously [2]. MSCs were maintained in a complete medium comprising of Dulbecco's modified Eagle's media (C/N 11885-084) supplemented with 20% fetal bovine serum (C/N 10270-098), 1% penicillin/streptomycin (C/N 15140-122) and 1% non-essential amino acid (C/N 11140-050) (all from Gibco, Life Technologies, Carlsbad, CA, USA). The medium was replaced every four days and cells were

maintained until passage 4 (P4) prior to their differentiation into neural lineages.

At P4, MSCs were differentiated into NPCs with NeuroCult® NS-A proliferation basal media (C/N 05771, STEMCELLS Technologies, Vancouver, BC, Canada) supplemented with either EGF + bFGF (Group A) or EGF + bFGF + IGF-1 (Group B). For negative control, the MSCs were differentiated in basal media without any growth factors (Group C). Cells were allowed to differentiate for 1 week at 37 °C with 5% carbon dioxide supply.

2.4. MicroRNA isolation and sample quality control

Total RNA including small RNA, 18 nucleotides upwards, was isolated and purified using miRNeasy mini kit (C/N 217004, Qiagen, Hilden, Germany) according to the manufacturer's protocol. Quantity and quality of the RNA were assessed by a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, Driesch, Germany) and a 2200 Agilent TapeStation System (Agilent Technologies, Santa Clara, CA, USA), respectively. We used the RNAs with integrity number equivalent (RIN^e) 9.0 and above. In all of our samples we also confirmed the presence of small RNA fractions. Only RNA samples with a 28S/18S proportion more than 1.2, detectable small RNA population and RIN^e more than 9.0 were used for microarray analysis. Purified RNA samples were aliquoted and stored at −80 °C until used.
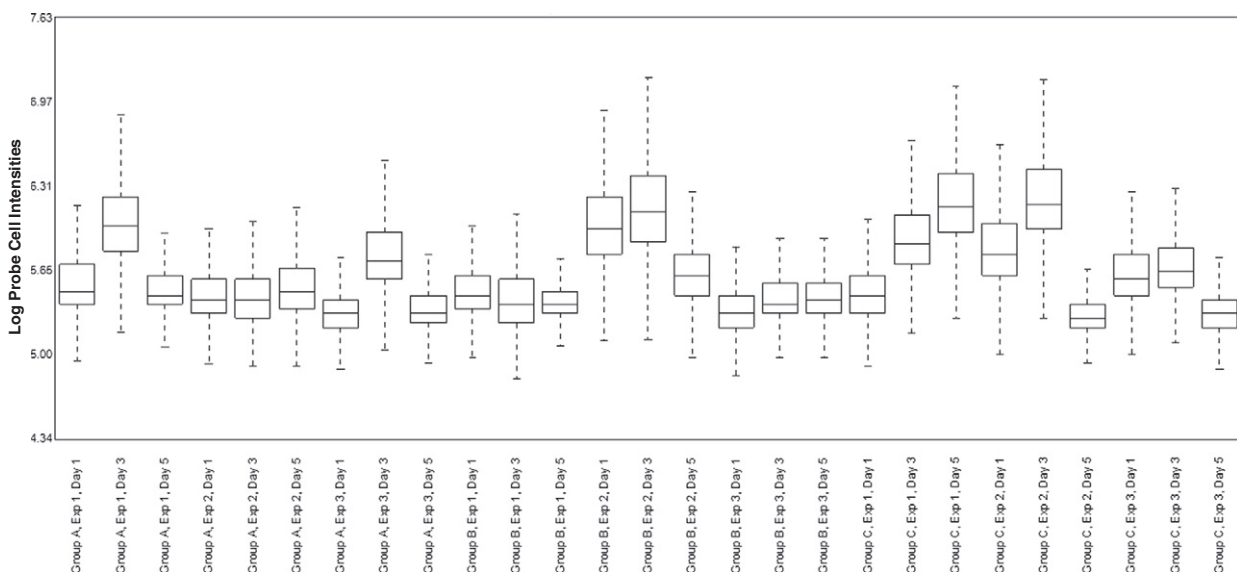
Fig. 2. Box plot generated from the probe cell intensity values directly from CEL file prior to normalization and summarization. Outliers are not shown and the values are log2.
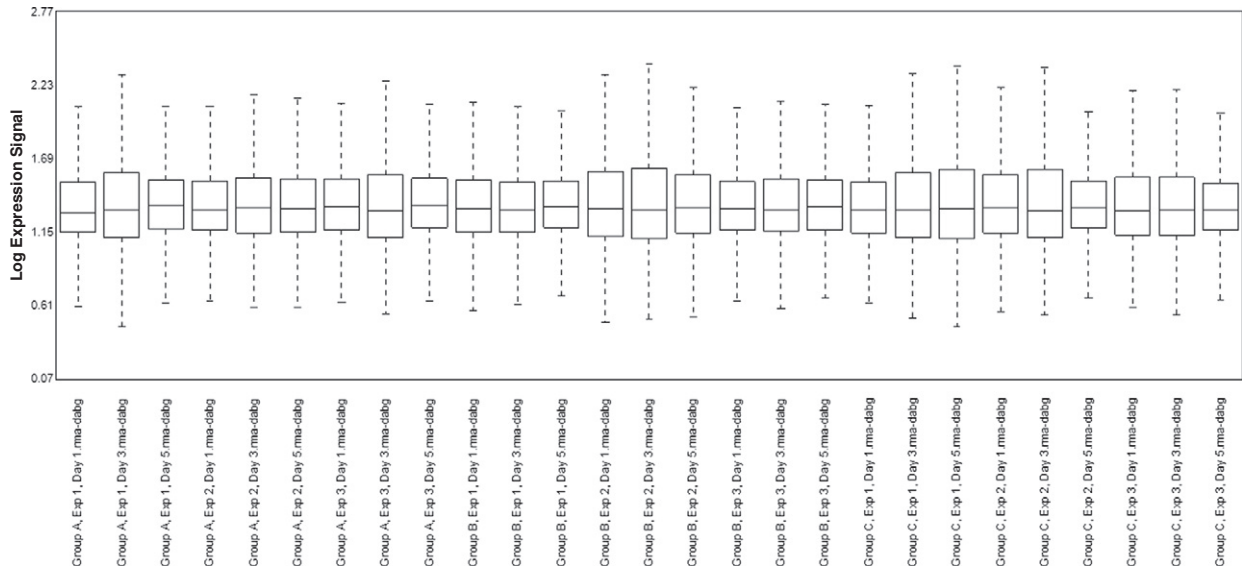
Fig. 3. Box plot generated from the probe set signal values that have been normalized by RMA and summarized. Outliers are not shown and the values are log2.

2.5. Microarray study design

Total RNAs from each group (Group A, Group B and Group C) were isolated at three different time intervals (24 h, 72 h and 120 h). Each sample was collected from three biological replicates of experiment. The RNA samples were subjected to microarray analysis using GeneChip miRNA 2.0 Arrays (C/N 901753, Affymetrix, Santa Clara, CA, USA).

2.6. RNA labeling and ELOSA assay

Total RNA (1000 ng) from all 27 samples was polyadenylated and conjugated to a biotin molecule using a FlashTag™ biotin HSR RNA labeling kit (vial 1–12) (C/N HSR10FTA, Genisphere, Hatfield, PA, USA) according to the manufacturer's instructions. Briefly, RNA samples were poly(A) tailed for 15 min at 37 °C with the presence of RNA spike control oligos (vial 8) and poly A tailing master mix consists of 10× reaction buffer (vial 1), 25 mM MnCl$_2$ (vial 2), ATP mix (vial

3) and PAP enzyme (vial 4). Then, the poly A tailed samples were conjugated with biotin molecule for 30 min at room temperature (25 °C) using 5× FlashTag biotin HSR ligation mix (vial 5) and T4 DNA ligase (vial 6). The reaction was stopped by adding HSR stop solution (vial 7).

Biotin-labeled samples (2 μL) were then subjected to Enzyme Linked Oligosorbent Assay (ELOSA) to confirm successful labeling. Briefly, samples were seeded into a flat bottom Immobilizer™ amino plate (C/N FT5ELOSA, Genisphere, Hatfield, PA, USA) pre-coated with ELOSA spotting oligos (vial 9) for 1 h at room temperature. Signal was developed by incubating each well with TMB substrate for 30 min. The blue substrate color was compared with ELOSA positive control (vial 10) and negative control. Samples with successful biotin labeling were proceeded to array hybridization.

2.7. Hybridization and scanning

Biotinylated RNA samples (21.5 μL) were added to hybridization cocktail comprising of 2× hybridization mix, 27.5% formamide (vial
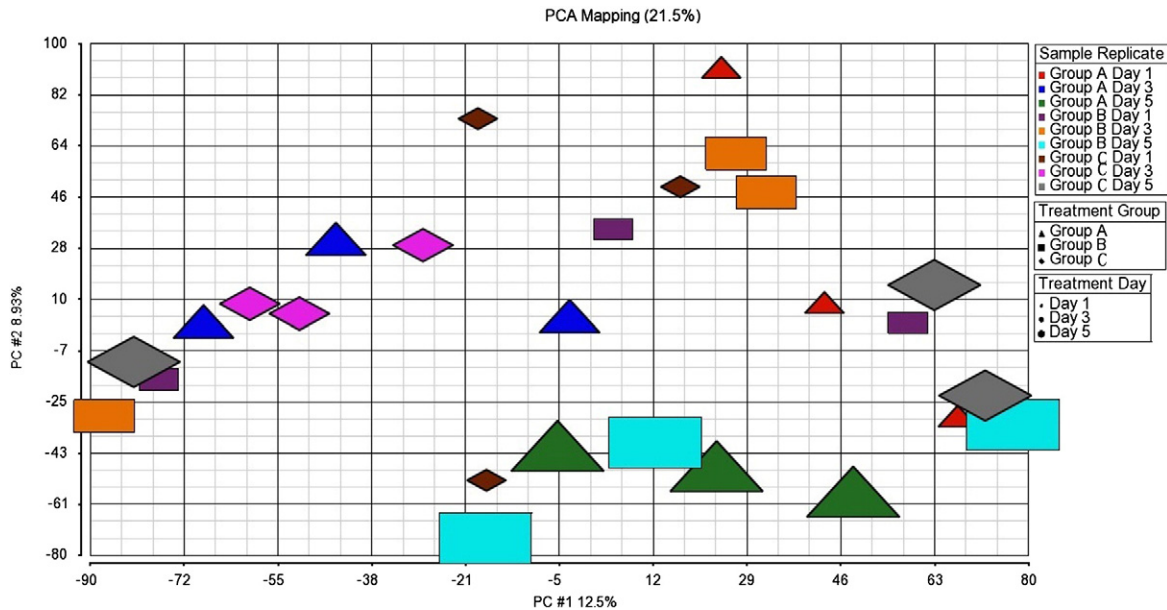

Fig. 4. Three-dimensional principal component analysis plot of all arrays colored and shaped according to sample replicate, treatment and day.

12), dimethyl sulfoxide, pre-heated $20\times$ eukaryotic hybridization controls (*bioB*, *bioC*, *bioD* and *creX*), 3 nM control oligo B2 and nuclease-free water (vial 11). The mixture was incubated at 99 °C for 5 min and then 45 °C for 5 min before injected into an array. Arrays were incubated in a hybridization oven for 16 h protected from light at 48 °C with a rotation speed of 60 rpm. After hybridization, the arrays were washed and stained according to the FS450_0003 fluidics protocol using Affymetrix GeneChip® Fluidics Station 450. The arrays were then scanned using a GeneChip® 3000 7G scanner.

2.8. Data normalization and basic analysis

Raw data files (CEL files) were generated for the 27 samples using Affymetrix GeneChip® Command Console® Software and subjected to quality control using microRNA QC Tool software. Hybridization and overall signal quality were determined by examining the spike-in control expression: *bioB*, *bioC*, *bioD* and *creX* from the lowest to the highest respectively (Fig. 1). Probe set intensities (CHP files) were obtained by

Robust Multi-array Average (RMA) background correction, data normalization, summarization and $\log_2$ transformation using Affymetrix Expression Console™ software (v1.4). Box plots indicating pre-normalization log probe cell intensity (Fig. 2) and post-normalization log expression signal (Fig. 3) were generated by Transcriptome Analysis Console software.

CHP files were then further analyzed by Partek® Genomics Suite software, version 6.6 (Partek Inc., St. Louis, MO, USA). Three dimensional principal component analysis (PCA) plot was generated to visualize data distribution and outliers (Fig. 4). PCA plot identified acceptable variability attributable to laboratory procedures across all arrays or biological replication of samples. Intra-group correlation of expression data shows 80–99% match.

2.9. Statistical data analysis

In order to cross-compare significant microRNA expression between treatment groups (A and B) and negative control Group C, statistical
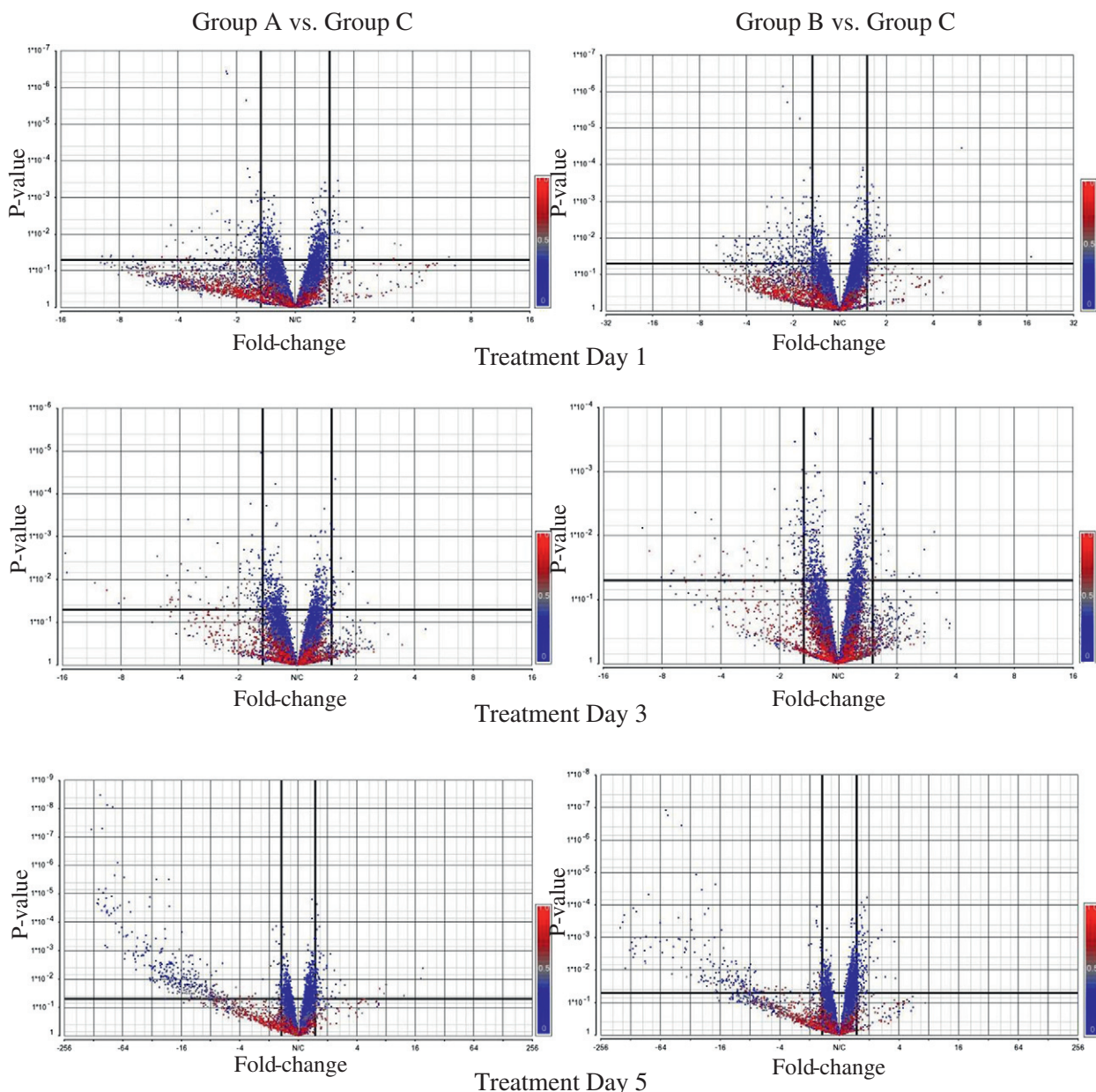

Fig. 5. Volcano plot representation of microarray data showing both significantly expressed genes and the magnitude of change.

significance analysis was performed using ANOVA. Significant data were presented in volcano plots (Fig. 5). Negative log10 p-value on y-axis indicates the significant level of each gene while the fold change (log base 2) on the x-axis indicates the relative gene expression between groups. Predicted target genes for the differentially expressed microRNAs were obtained from miRWalk online database (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html).

3. Discussion

Here we elucidate a unique microRNA expression dataset from rats' bone marrow MSC-derived NPCs. These datasets may provide clues to further our understanding about the mechanism underlying MSCs differentiation into neural lineage. This knowledge will lead to enhanced differentiation of MSCs into NPCs and it could be therapeutically beneficial in the future for neurodegenerative diseases. The expression data described here is of highest quality with multi-level QC protocols from sample preparation till statistical analysis. This data has been used in studies published recently [1] in journal with open access and high impact. We believe that this data will be of great value to future investigators with similar research interests.

Conflict of interest

None.

Acknowledgments

References

[1] T.J. Huat, A.A. Khan, J.M. Abdullah, F.M. Idris, H. Jaafar, MicroRNA expression profile of neural progenitor-like cells derived from rat bone marrow mesenchymal stem cells under the influence of IGF-1, bFGF and EGF. Int. J. Mol. Sci. 16 (5) (2015) 9693–9718.

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