Therapeutic Potential of Human Nasal Inferior Turbinate-Derived Stem Cells: Microarray Analysis of Multilineage Differentiation

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Human stem cells · Differentiation · Osteogenesis · Chondrogenesis · Neurogenesis · Gene expression · Microarrays · Gene ontology terms

Abstract
Introduction: Human nasal inferior turbinate-derived stem cells (hNTSCs) are attractive sources of adult stem cells for medical application because they can be easily obtained and cultivated with a highly proliferative capacity. The ability of hNTSCs to differentiate into chondrocytes, osteocytes, and neural cells makes them potential replacement therapeutic candidates in intractable diseases. Nevertheless, detailed expression pattern of genes associated with trilineage differentiation (osteogenesis, chondrogenesis, and neurogenesis) in hNTSCs has not been revealed yet. Methods: In this study, we aimed to evaluate gene expression patterns of various transcription factors and marker genes associated with a particular lineage (osteogenesis, chondrogenesis, and neurogenesis) of differentiation of hNTSCs by DNA microarrays. Results: In microarrays, 36 transcripts such as E2F transcription factor 1, activating transcription factor 5, and AKR1B10 were upregulated and 36 transcripts such as CA9, PPFA4, HAS2, and COL4A4 were downregulated in osteogenically differentiated hNTSCs. In chondrogenically differentiated hNTSCs, 3 transcripts (NUDT14, CPA4, and heparin-binding epidermal growth factor-like growth factor) were upregulated and 82 transcripts such as PTGS1, CLEC2D, and TET1 were downregulated. In neurally differentiated hNTSCs, 61 transcripts such as insulin-like growth factor-binding protein-1, nerve growth factor receptor, FGF1, OLFML1, and EPGN were upregulated and 98 transcripts such as ACAN, RUNX2, and C21orf96 were downregulated. In gene ontology (GO) analysis, cell signal-related GO terms were highly expressed. By contrast, catalysis GO terms and GO terms related to oxidoreductase were overrepresented in chondrogenically differentiated hNTSCs and osteogenically differentiated hNTSCs, respectively. Conclusion: Considering overall results, hNTSCs-specific genetic information may promote further studies on intracellular mechanisms defining key features of these cells.

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Introduction

Mesenchymal stem cells (MSCs) are adult stem cells with the potential to differentiate into various cell lineages [1]. They can be isolated from various tissues of adults [1, 2]. Previously, we have shown that MSCs derived from nasal mucosa (inferior turbinate) (hNTSCs) have a high proliferation potential. In addition, characteristics of hNTSCs do not change regardless of cell passage number [1]. Moreover, hNTSCs show superior potential to differentiate into osteocytes and chondrocytes. These cells have better osteogenic potential regardless of accessorial osteogenic factors, including vitamin D3 and BMP-2, in comparison with MSCs isolated from bone marrow or adipose tissues [3]. These cells can differentiate easily into chondrocytes by in vitro culture without co-culture of MSCs and chondrocytes which can enhance matrix deposition [4]. MSCs are considered as important sources for cellular therapy due to their immunologically privileged properties. Additionally, the isolation of MSCs from tissues normally removed during surgery and storage of them would have significant advantage for patients with various diseases [5]. Although hNTSCs provide a novel opportunity for regenerative medicine, in otorhinolaryngologic parts, core genes during the differentiation process of hNTScs have not been evaluated sufficiently. Thus, the objective of this study was to determine differentiation phenotypes of hNTSCs under appropriate differentiation-induction media (osteogenic, chondrogenic, and neurogenic differentiation) and perform microarray-based gene expression profiling to evaluate these differentiation phenotypes of cell populations.

Materials and Methods

Statement of Ethics

hNTSCs were isolated from patients who underwent an inferior turbinate surgery. Isolation procedures were approved by the Institutional Review Board of Seoul St. Mary’s Hospital, the Catholic University of Korea (KC08TISS0341). Written informed consent was obtained from the subjects.

Cell Isolation

The inferior turbinate tissue obtained after surgery was washed 3 to 5 times with an antibiotic-antimycotic solution (Gibco, Gaithersburg, MD, USA) and 3 times with phosphate buffered saline (PBS). It was then cut into 0.5-mm³ pieces. These pieces were placed into a culture dish. The dish was covered with a sterilized glass cover slide. Alpha-MEM (WELGENE, Gyeongsan, Korea) containing 10% fetal bovine serum (FBS; WELGENE) and 1% antibiotic-antimycotic solution (Gibco) was added. It was changed every 2 days. After 15 days of culture at 37°C in a 5% CO₂ incubator, the glass cover slide was removed and tissues floating in the culture media were removed by washing. hNTSCs attached to the bottom of the culture dish were then detached using 1 mL of 0.05% trypsin in 1 mM EDTA. After isolation, hTMSCs were seeded at an initial cell density of 1 × 10⁶ cells/cm² and amplified in a monolayer culture media. hNTSCs were cultured for 3 passages to evaluate their multidifferentiation potential.

Characterization of Surface Markers on hNTSCs by Flow Cytometry

To investigate the expression of cell surface marker proteins in hNTSCs at passage 3, cells were detached and labeled with FITC- or PE-coupled anti-human antibodies (CD14, CD19, CD29, CD34, CD73, CD90, and HLA-DR; BD Biosciences, San Jose, CA, USA). Ten thousand labeled cells were measured using a FACS calibur flow cytometer (Becton Dickinson, Franklin lakes, NJ, USA). Results were analyzed with a CellQuest software (Becton Dickinson).

Multilineage Differentiation Potential of hNTSCs

To investigate the osteogenic potential of hNTSCs, cells were cultured in a low-glucose DMEM supplemented with 10% (v/v) FBS, 0.1 μM dexamethasone, 10 mM β-glycerophosphate, 50 μM ascorbate-2-phosphate, 100 U/mL penicillin, and 100 μg/mL streptomycin. Culture media were changed twice every week for 4 weeks. Differentiation to osteogenic lineages was induced according to the procedures described by Hwang et al. [1]. A 3-dimensional culture system was used for chondrogenic differentiation with open-cell polylactic acid (BD Biosciences). After 2 × 10⁶ cells were seeded onto the scaffold, cells were cultured in chondrogenic induction media consisting of DMEM supplemented with 10% FBS, 100 nM dexamethasone, 50 μM ascorbic acid-2-phosphate (Sigma, St. Louis, MO, USA), 50 mg/mL insulin-transferrin-selenium, 1 mM sodium selenite, 1 mM sodium pyruvate (Gibco), 40 μM proline, and 2 mM L-glutamine added to chondrogenic supplements TGF-β1 (10 ng/mL; Sigma) and IGF-1 (10 ng/mL; Sigma). Culture media were changed twice every week for 2 weeks. To induce neurogenic differentiation, hNTSCs were seeded onto 4-well chamber slides (1 × 10⁴ cells/well; Nalgene Nunc International, Rochester, NY, USA) and cultivated in neurobasal media. Cultivated cells were suspended in DMEM/F-12 (Gibco) with 1 mL B-27 supplement, neurogenic supplements (10 ng/mL gial-derived neurotrophin factor [Invitrogen, Carlsbad, CA, USA], 10 ng/mL neurotrophin-3 [Invitrogen], 10 ng/mL brain-derived neurotrophin factor [Invitrogen], 2 mM L-glutamine), 0.5% heparin, and 1% penicillin-streptomycin (Gibco) for 2 weeks. Culture medium was refreshed every 2 days.

Immunocytochemistry of Differentiated hNTSCs

The differentiated hNTSCs were fixed with 4% paraformaldehyde in PBS for 20 min, and nonspecific binding sites were blocked by a 30-min incubation in PBS containing 0.5% Triton X-100 (Pro-mega Co. Medison, WI, USA) at room temperature, then stained to analyze cytologically with Safranin O and Alizarin red for chondrocyte and osteocyte differentiation. For immunocytofluorescence staining, it is following by antigen retrieval with proteinase K (Abcam) to expose the antigenic sites. After blocking with 5% normal goat serum, cells were each incubated with primary antibodies against NeuN (Abcam) and β-tubulin (Abcam) at room temperature overnight for double staining and washed thrice with 0.01 mol/L PBS. After incubating with secondary antibodies, Alexa 488
Gene Expression Profile

In osteogenic differentiated hNTSCs, 36 transcripts such as E2F transcription factor 1 (E2F1), activating transcription factor 5 (ATF5), and AKR1B10 were upregulated and 36 transcripts such as CA9, PPFIA4, HAS2, and COL4A4 were downregulated. In chondrogenic differentiated hNTSCs, 3 transcripts (NUDT14, CPA4, and heparin-binding epidermal growth factor-like growth factor [HBEGF]) were upregulated and 82 transcripts such as PTGS1, CLEC2D, and TET1 were downregulated. In neural differentiated hNTSCs, 61 transcripts such as insulin-like growth factor-binding protein (IGFBP)-1, nerve growth factor receptor (NGFR), FGF1, OLFML1, and EPGN were upregulated and 98 transcripts such as ACAN, RUNX2, and C21orf96 were downregulated. These up- and downregulated genes in trilineage differentiated hNTSCs are listed in Tables 1–3 and online suppl. Table 1; see www.karger.com/doi/10.1159/000516016 for all online suppl. material, (fold change in expression). Ad-

Results

Isolation and Characterization of hNTSCs through Flow Cytometry

Fibroblast-like cells appeared within 3 days, adhered to the culture dish, and formed into a single layer. Flow cytometric analysis was performed for cell surface markers of in vitro-cultured hNTSCs. Results revealed that these cells did not show any expression of hematopoietic cell markers (CD14, CD19, CD34, or HLA-DR). However, they showed expression of MSC markers (CD29, CD73, and CD90) (Fig. 1).

Immunocytochemistry of Differentiated hNTSCs

The cells exposed to osteogenic medium showed direct evidences of calcium mineralization seen by Alizarin Red staining. Cells after neurogenic induction for 14 days were analyzed with immunocytochemistry and the expression of nestin and β-tubulin was detected. Safranin O binds specifically detected the proteoglycan in chondrogenic differentiated hNTSCs (Fig. 2).
Additionally, principal component analysis plot for comparing differences of cell groups according to differentiation and a heatmap for comparing genes differentially expressed in undifferentiated and trilineage differentiated hNTSCs were prepared. Results are shown in Figures 3, 4.

**Gene Ontology Analysis**

Major over- and underrepresented gene ontology (GO) classes are shown in Tables 4–6. In neural differentiated hNTSCs, major GO classes of cytokine or chemokine were abundant. In chondrogenic differentiated hNTSCs, catalysis GO terms were overrepresented. In osteogenic differentiated hNTSCs, GO terms related to oxidoreductase were overrepresented.

**Discussion**

Results of the present study verified specific gene expression patterns in hNTSCs according to trilineage differentiation. These results were compared to expression profiles of undifferentiated hNTSCs as a reference. Expression profiles of osteo-, chondro-, and neuro-differentiated hNTSCs were found to be different from each other. Based on expression profile analysis, many genes were shown to be up- or downregulated. Furthermore, GO analyses of regulated gene products showed over- or underexpressed GO classes. These results provide a first step for revealing core molecules associated with characteristics of hNTSCs.

Microarrays have become vital tools for detecting the expression of thousands of genes and for identifying differentially expressed genes. They enable us to explain and compare gene expression patterns of cells during or after differentiation. Based on microarray data, we can comprehend mechanisms controlling the expression of individual cells’ characteristics. Some microarray studies have been conducted to check new specific markers and investigate the origin of various diseases including cancers [6–8].

In the otolaryngologic part, partial turbinate resection is one of the most popular and efficient surgery for reduc-

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**Fig. 2.** Histologic analysis of hTMSCs 2–3 weeks after trilineage differentiation. The cells exposed to chondrogenic (a) and osteogenic (b) medium showed direct evidences of proteoglycan and calcium mineralization seen by Safranin O staining and Alizarin Red staining. Cells after neurogenic induction expressed nestin and β-tubulin (c). hTMSCs, human turbinate mesenchymal stromal cells.
Table 1. Identification of genes differentially expressed during chondrogenesis

| RefSeq ID   | Gene name                                                                 |
|-------------|---------------------------------------------------------------------------|
| **Upregulated genes**                                                                 |
| NC_000014.9 | Nudix-type motif 14                                                       |
| NC_000007.14| Carboxypeptidase A4                                                        |
| NC_000005.10| HBEGF                                                                     |
| **Downregulated genes**                                                                 |
| NC_000009.12| Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) |
| NC_000012.12| C-type lectin domain family 2 member D                                    |
| NC_000077.6 | SH3 and cysteine-rich domain 2                                             |
| NC_00019.18 | Hypothetical protein LOC100128567                                         |
| NC_00010.11 | Tet methylcytosine dioxygenase 1                                          |
| NC_00003.12 | Ceruloplasmin (ferroxidase)                                                |
| NC_00007.14 | Hypoxia inducible lipid droplet associated                                |
| AK094436.1 | Uncharacterized LOC284219                                                 |
| NP_775819.1 | Ensembl 59 human genes, GRCh37 assembly-ZNF584                            |
| NC_00007.14 | Leptin                                                                    |
| XM_01123368 | Similar to cholinergic receptor, muscarinic 3                              |
| NC_00016.10 | Proline-rich transmembrane protein 2                                      |
| NC_00006.12 | POU class 5 homeobox 1                                                    |
| NC_00005.10 | Family with sequence similarity 153, member B                             |
| NC_00005.10 | NLR family, apoptosis inhibitory protein                                  |
| NC_00006.12 | Forkhead box Q1                                                           |
| NC_00005.10 | Liver-expressed antimicrobial peptide 2                                   |
| NC_00014.9 | FBJ murine osteosarcoma viral oncogene homolog                            |
| NC_00019.10 | Intercellular adhesion molecule 5, telencephalin                           |
| NC_00014.7 | Uncharacterized LOC440200                                                 |
| NC_00022.11 | Galactose-3-O-sulfotransferase 1                                           |
| NC_00007.14 | CCZ1 homolog B, vacuolar protein trafficking and biogenesis associated    |
| NC_00007.14 | Leucine-rich single-pass membrane protein 1                               |
| NC_00022.11 | Uncharacterized LOC729461                                                 |
| NC_00005.10 | Phosphodiesterase 8B                                                      |
| NC_00010.11 | RUN and FYVE domain containing 2                                           |
| NC_00009.11 | Uncharacterized MGC24103                                                  |
| NC_00019.10 | Zinc finger protein 615                                                    |
| NC_00011.10 | H19, imprinted maternally expressed transcript                            |
| NC_00011.10 | KCNQ1 opposite strand/antisense transcript 1                              |
| NC_00016.10 | Guanine nucleotide binding protein                                         |
| NC_00011.10 | Mucin 5AC, oligomeric mucus/gel-forming                                   |
| NC_00020.11 | Family with sequence similarity 65, member C                              |
| NC_00017.11 | Ring finger protein 11                                                    |
| NC_00001.11 | Phosphodiesterase 4B, cAMP-specific                                       |
| NC_00009.12 | Carbonic anhydrase IX                                                     |
| NC_00010.9 | Uncharacterized protein BC008131                                          |
| NC_00017.11 | RAS, dexamethasone-induced 1                                              |
| NC_00002.12 | Ankyrin repeat domain 36B                                                |
| NC_00006.12 | Tenascin XB                                                               |
| NC_00007.14 | WD repeat domain 86                                                       |
| AL110203.1  | Uncharacterized LOC158863                                                 |
| NC_00010.11 | Uncharacterized LOC399715                                                |
| NC_00007.14 | Nicotinamide phosphoribosyltransferase                                    |
| NC_00009.12 | Uncharacterized protein PRO2852                                           |
| NC_00006.12 | Suppressor APC domain containing 1                                        |
| NC_00017.11 | Gasdermin B                                                               |
| NC_00001.11 | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) |
| NC_00015.10 | Long intergenic nonprotein coding RNA 597                                |
| NC_00004.10 | Uncharacterized LOC100131829                                              |
ing nasal symptoms. MSCs derived from the inferior tubinate can be obtained in ease and abundance. Due to this feature, hNTSCs could serve as important resources of adult stem cells. However, characteristics and differentiation of hNTSCs using genome-wide research techniques have not been reported yet. Studies on MSC derived from different tissues have shown a phenotype analogous to each other. Nevertheless, considerable differences in the molecular phenotype among MSCs from different tissues have been reported, showing ontological and functional differences [9]. Thus, it would be important to perform genome-wide gene expression profiling and GO analysis for hNTSCs. Thus, this is the first study to obtain gene expression profiles of hNTSCs through microarray.

HBEGF is a mitogenic and chemotactic molecule related to tissue repair and other tissue-modeling phenomena. Krampera et al. [10] have found that HBEGF can increase the proliferation of bone marrow MSCs but prevent chondrogenic differentiation reversibly. However, in our study, hNTSCs showed increasing HBEGF expression during chondrogenic differentiation. Transforming growth factor beta can induce extracellular matrix proteins during chondrogenic differentiation in vitro. In

| RefSeq ID          | Gene name                                      |
|--------------------|------------------------------------------------|
| NC_000006.12       | Tudor domain containing 6                     |
| NC_000007.14       | Cyclin D binding myb-like transcription factor 1 |
| NC_000002.11       | Uncharacterized LOC100128563                  |
| NC_000006.12       | Wilms tumor 1-associated protein              |
| NC_000011.10       | Dopamine receptor D4                          |
| NC_000020.10       | Uncharacterized LOC29053                      |
| NC_000023.11       | Apelin                                         |
| NC_000003.12       | Sarcolemma-associated protein                 |
| BX647230.1         | Uncharacterized LOC399832                     |
| NC_000002.12       | Frizzled-related protein                      |
| NC_000019.10       | Cartilage intermediate layer protein 2        |
| NC_000008.11       | Metastasis suppressor 1                       |
| NC_000014.9        | RAS (RAD and GEM)-like GTP binding 2          |

Nudix, nucleoside diphosphate-linked moiety X; HBEGF, heparin-binding epidermal growth factor-like growth factor.

Table 1 (continued)
### Table 2. Identification of genes differentially expressed during osteogenesis

| RefSeq ID        | Gene name                                                      |
|------------------|---------------------------------------------------------------|
| **Upregulated genes** |                                                               |
| NC_000009.12     | Mmicroseminoprotein, prostate associated                      |
| NC_000020.11     | E2F transcription factor 1                                    |
| NC_000008.11     | Scavenger receptor class A, member 5                          |
| NC_000019.10     | Activating transcription factor 5                             |
| NC_000012.12     | LY6/PLAUR domain containing 1                                 |
| NC_000003.12     | Acyl-CoA oxidase 2, branched chain                            |
| NC_000015.10     | Aldehyde dehydrogenase 1 family, member A3                   |
| NC_000003.12     | Family with sequence similarity 107, member A                 |
| NC_000012.12     | DEAD (Asp-Glu-Ala-Asp) box polypeptide 54                    |
| NC_000002.12     | Vitrin                                                        |
| NC_000011.10     | Matrix metallopeptidase 1                                    |
| NC_000012.12     | G protein-coupled receptor, class C, group 5, member A        |
| NC_000014.9      | Nudix-type motif 14                                           |
| NC_000002.12     | Kynureninase                                                  |
| NC_000001.11     | Vav 3 guanine nucleotide exchange factor                      |
| NC_000019.10     | Anti-silencing function 1B histone chaperone                 |
| NC_000016.10     | Periplakin                                                    |
| NC_000010.11     | Interferon-induced protein with tetratricopeptide repeats 3  |
| NC_000017.11     | Chemokine (C-X-C motif) ligand 16                             |
| NC_000007.14     | Carboxypeptidase A4                                           |
| NC_000007.14     | Aldo-keto reductase family 1, member B10 (aldose reductase)  |
| NC_000023.11     | Retinoic acid-induced 2                                       |
| NC_000006.12     | Transcription factor AP-2 beta (activating enhancer binding protein 2 beta) |
| NC_000014.9      | Deiodinase, iodothyronine, type II                            |
| NC_000007.14     | STEAP family member 4                                         |
| NC_000019.10     | Aldehyde dehydrogenase 16 family, member A1                  |
| NC_000009.12     | Phytanoyl-CoA dioxygenase domain-containing 1                |
| NC_000023.11     | Nuclear receptor subfamily 0, group B, member 1               |
| NC_000004.12     | Toll-like receptor 3                                          |
| NC_000009.12     | Glycine dehydrogenase (decarboxylating)                      |
| NC_000016.10     | GINS complex subunit 2 (Psf2 homolog)                        |
| NC_000011.10     | Purinergic receptor P2Y, G-protein coupled, 2                |
| NC_000017.11     | ATP-binding cassette, subfamily A (ABC1), member 6            |
| **Downregulated genes** |                                                               |
| NC_000009.12     | Carbonic anhydrase IX                                         |
| NC_000001.11     | Protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4 |
| NC_000008.11     | Hyaluronan synthase 2                                         |
| NC_000002.12     | Collagen, type IV, alpha 4                                    |
| NC_000007.14     | Leptin                                                       |
| NC_000020.11     | Fer-1-like family member 4, pseudogene (functional)          |
| NC_000007.14     | Hypoxia inducible lipid droplet-associated                    |
| NC_000001.11     | Dual specificity phosphatase 5 pseudogene 1                  |
| NC_000009.12     | Very low-density lipoprotein receptor                        |
| NC_000009.12     | Prostaglandin D2 synthase 21 kDa (brain)                     |
| NC_000005.10     | Family with sequence similarity 153, member B                |
| NC_000014.7      | Uncharacterized LOC440200                                     |
| NC_000012.12     | Cysteine and glycine-rich protein 2                          |
| NC_000017.11     | Serine/arginine-rich splicing factor 1                        |
| NC_000001.11     | Thioredoxin interacting protein                               |
| NC_000017.11     | Arachidonate 15-lipoxygenase, type B                          |
| NC_000012.12     | C-type lectin domain family 2, member D                      |
| NC_000017.11     | Asialoglycoprotein receptor 1                                 |
| NC_000011.10     | KCNQ1 opposite strand/antisense transcript 1 (nonprotein coding) |
| NC_000006.12     | Collagen, type XXI, alpha 1                                   |
chondrocyte progenitor cells stimulated by transforming growth factor beta, HBEGF was strongly expressed during induction, suggesting that HBEGF could be important in extracellular matrix manufacture as with cell function [11]. Our results showed that hTMSCs after differentiation were most similar to chondrocyte progenitor cells.

This could explain why HBEGF is an important role during the chondrogenic differentiation of hNTSCs. ATF5 is a member of the ATF/CREB family of beta-ZIP transcription factors. It is strongly expressed in various neoplasms. It also controls stem cell functions [12]. During osteogenic differentiation of MSCs from human adipose tissue, ATF5 was highly expressed, reaching a peak of expression at the stage of bone mineralization. Vicari et al. [13] have suggested that ATF5 could play an interesting regulatory role during osteogenesis. These results were similar to our results showing that ATF5 was expressed highly during osteogenic differentiation.

E2F1 functions as a DNA-binding domain of pRb. Signaling via the interaction of pRb-E2F is considered to be associated with cell cycle regulation, cell fate, and differentiation of MSCs [14]. pRB is an activator of tissue-specific gene expression along diverse lineages including osteoblast differentiation [15]. In addition, the transcription start site of the ATF5 gene with promoter activity retaining has potential binding sites for several transcrip-

Table 2 (continued)

| RefSeq ID       | Gene name                                      |
|-----------------|------------------------------------------------|
| NC_000023.11    | Apelin                                         |
| NC_000006.12    | Vascular endothelial growth factor A           |
| NC_000017.11    | Ring finger protein 112                        |
| NC_000015.10    | Prader Willi/Angelman region RNA, SNRPN neighbor|
| NC_000001.11    | Uncharacterized LOC100131564                   |
| NC_000004.12    | Chromosome 4 open reading frame 47             |
| NC_000002.12    | Ankyrin repeat domain 36B                      |
| NC_000009.12    | Prune homolog 2 (Drosophila)                   |
| NC_000013.11    | Periostin, osteoblast-specific factor           |
| NC_000022.11    | Galactose-3-O-sulfotransferase 1               |
| NC_000006.12    | Wilms tumor 1-associated protein               |
| NC_000019.10    | Cartilage intermediate layer protein 2         |
| NC_000019.10    | Intercellular adhesion molecule 5, telencephalin|
| NC_000021.9     | Junctional adhesion molecule 2                 |

Nudix, nucleoside diphosphate-linked moiety X.
### Table 3. Identification of genes differentially expressed during neurogenesis

| RefSeq ID     | Gene name                                                                 |
|---------------|---------------------------------------------------------------------------|
| Upregulated genes |                                                                            |
| NC_000002.12  | LON peptidase N-terminal domain and ring finger 2                           |
| NC_000004.12  | Annexin A10                                                               |
| NC_000004.12  | Heparan sulfate (glucosamine) 3-O-sulfotransferase 1                       |
| NC_000002.12  | Adapter-related protein complex 1, sigma 3 subunit                         |
| NC_000005.10  | Family with sequence similarity 169, member A                              |
| NC_000009.12  | TEK tyrosine kinase, endothelial                                           |
| NC_000007.14  | Insulin-like growth factor binding protein 1                               |
| NC_000017.11  | Chemokine (C-X-C motif) ligand 16                                          |
| NC_000007.14  | Fibrinogen-like 2                                                          |
| NC_000004.12  | Chemokine (C-X-C motif) ligand 6                                           |
| NC_000050.10  | Proprotein convertase subtilisin/kexin type 1                              |
| NC_000012.12  | Parathyroid hormone-like hormone                                          |
| NC_000001.11  | Lipid phosphate phosphatase-related protein type 4                         |
| NC_000004.12  | Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) |
| NC_000017.11  | Nefdin 1                                                                   |
| NC_000012.12  | Retinol dehydrogenase 5 (11-cis/9-cis)                                     |
| NC_000011.10  | Chordin-like 2                                                             |
| NC_000001.11  | Hydroxysteroid (11-beta) dehydrogenase 1                                   |
| NC_000005.10  | Cytoplasmic FMR1 interacting protein 2                                     |
| NC_000002.12  | Sperm-associated antigen 16                                                |
| NC_000004.12  | Chemokine (C-X-C motif) ligand 2                                           |
| NC_000009.12  | Tumor necrosis factor (ligand) superfamily, member 15                     |
| NC_000009.12  | Microsminoprotein, prostate associated                                     |
| NC_000001.11  | Regulatory subunit of type II PKA R-subunit (RIIa) domain containing 1     |
| NC_000012.12  | Serine dehydratase-like                                                   |
| NC_000002.12  | Kynurenicase                                                               |
| NC_000017.11  | Chemokine (C-C motif) ligand 7                                             |
| NC_000011.10  | Olfactomedin-like 1                                                        |
| NC_000003.12  | Acyl-CoA oxidase 2, branched chain                                         |
| NC_000020.11  | Potassium voltage-gated channel, modifier subfamily S, member 1           |
| NC_000010.11  | Internexin neuronal intermediate filament protein, alpha                   |
| NC_000004.12  | Epithelial mitogen                                                         |
| NC_000014.9   | Long intergenic nonprotein coding RNA 643                                |
| NC_000001.11  | Chitinase 3-like 2                                                         |
| NC_000003.12  | Claudin 1                                                                  |
| NC_000013.11  | Glypcan 5                                                                  |
| NC_000014.9   | Somatostatin receptor 1                                                    |
| NC_000017.11  | Nerve growth factor receptor                                               |
| NC_000017.11  | Family with sequence similarity 20, member A                               |
| NC_000012.12  | Spexin hormone                                                             |
| NC_000017.11  | Chemokine (C-C motif) ligand 2                                             |
| NC_000007.14  | Tissue factor pathway inhibitor 2                                          |
| NC_000016.10  | Chromosome 16 open reading frame 89                                       |
| NC_000004.12  | Chemokine (C-X-C motif) ligand 3                                           |
| NC_000007.14  | Carboxypeptidase A4                                                        |
| NC_000007.14  | Calcium channel, voltage-dependent, alpha 2/delta subunit 1               |
| NC_000003.12  | Solute carrier family 7, member 14                                         |
| NC_000001.11  | Vascular cell adhesion molecule 1                                         |
| NC_000018.10  | Collectin sub-family member 12                                             |
| NC_000005.10  | Fibroblast growth factor 1 (acidic)                                        |
| NC_000011.10  | Dedicator of cytokinesis 1 pseudogene                                      |
| NC_000012.12  | TAP binding protein-like                                                  |
| NC_000004.12  | TLR3                                                                       |
| NC_000011.10  | Midkine (neurite growth-promoting factor 2)                                |
Table 3 (continued)

| RefSeq ID     | Gene name                                                                 |
|---------------|---------------------------------------------------------------------------|
| NC_000010.11  | Interferon-induced protein with tetratricopeptide repeats 3              |
| NC_000008.11  | Musculin                                                                  |
| NC_00020.11   | Peptidase inhibitor 3, skin-derived                                      |
| NC_000007.14  | Interleukin 6                                                             |
| NC_00011.10   | Insulin-like growth factor 2                                              |
| NC_00010.11   | Zinc finger protein 91 pseudogene                                        |
| NC_00001.11   | Glutamate-ammonia ligase                                                 |
| NC_00011.10   | H19, imprinted maternally expressed transcript (nonprotein coding)      |
| NC_00012.12   | Taste receptor, type 2, member 3                                          |
| NC_00015.10   | Aggrecan                                                                  |
| NC_00016.10   | Metallothionein 1X                                                        |
| NC_00009.12   | Uncharacterized protein PRO2852                                           |
| NC_00007.14   | Leptin                                                                    |
| NC_00003.12   | Sarcolemma associated protein                                             |
| NC_00017.11   | Ankyrin repeat and FYVE domain containing 1                               |
| NC_00005.10   | Family with sequence similarity 153, member B                             |
| NC_00001.11   | Cell adhesion molecule 3                                                  |
| NC_00019.10   | Cartilage oligomeric matrix protein                                        |
| NC_00013.11   | Collagen alpha-1(II) chain-like                                           |
| AB007954.1    | Uncharacterized LOC57235                                                  |
| NC_00022.11   | POM121 transmembrane nucleoporin-like 8 pseudogene                       |
| NC_00006.12   | FK506 binding protein 5                                                   |
| NC_00020.11   | Juncophilin 2                                                             |
| NC_00007.14   | Pyruvate dehydrogenase kinase, isozyme 4                                  |
| NC_00017.11   | Hepatic leukemia factor                                                   |
| NC_00019.10   | Cytokine receptor-like factor 1                                           |
| NC_00008.11   | Hair growth associated                                                    |
| NC_00017.11   | Noggin                                                                    |
| NC_00010.11   | Tet methylcytosine dioxygenase 1                                          |
| NC_007869.1   | Chemokine (C-X-C motif) receptor 7                                        |
| NC_00010.11   | Calcium channel, voltage-dependent, beta 2 subunit                        |
| NC_00017.11   | Chromosome 17 open reading frame 67                                       |
| NC_00010.11   | Sorbin and SH3 domain containing 1                                        |
| NC_00017.11   | Arachidonate 15-lipoxygenase, type B                                      |
| NC_00001.11   | HNRNPU antisense RNA 1                                                    |
| NC_00023.11   | TSC22 domain family, member 3                                             |
| NC_00022.11   | POM121 transmembrane nucleoporin-like 1, pseudogene                      |
| NC_00002.12   | Frizzled-related protein                                                  |
| NC_00002.12   | Methyltransferase like 21A                                                |
| NC_00006.12   | POU class 5 homeobox 1                                                    |
| NC_00004.10   | Uncharacterized LOC100131829                                             |
| NC_00014.9    | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), |
| NC_00010.11   | Nebulette                                                                 |
| NC_00013.11   | Regulator of cell cycle                                                   |
| NC_00017.11   | Coronin 6                                                                 |
| NC_00006.12   | Wilms tumor 1 associated protein                                          |
| NC_00011.10   | Platelet derived growth factor D                                          |
| NC_00021.9    | RUNX1 intronic transcript 1                                               |
| AL110203.1    | Uncharacterized LOC158863                                                 |
| NC_000007.1   | SND1 intronic transcript 1                                                |
| NC_00017.11   | Serine/arginine-rich splicing factor 1                                    |
| NC_00008.11   | PHD finger protein 20-like 1                                              |
| NC_00011.10   | Zinc finger and BTB domain containing 16                                  |
| NC_00006.12   | MyoD family inhibitor                                                    |
Table 3 (continued)

| RefSeq ID | Gene name |
|-----------|-----------|
| NC_000007.14 | WD repeat domain 86 |
| NC_000020.11 | Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein |
| NC_000020.11 | Acyl-CoA synthetase short-chain family member 1 |
| NC_000009.11 | Uncharacterized MGC24103 |
| NC_000005.10 | CD14 molecule |
| NC_000015.10 | Putative ubiquitin-conjugating enzyme E2Q2-like protein |
| NC_000020.11 | V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B |
| NC_000006.12 | Runt-related transcription factor 2 |
| NC_000007.14 | Leucine-rich single-pass membrane protein 1 |
| NC_000011.10 | BH3-like motif containing, cell death inducer |
| NC_000003.12 | Long intergenic nonprotein coding RNA 312 |
| NT_113901.1 | Uncharacterized LOC645566 |
| NC_000005.10 | Liver expressed antimicrobial peptide 2 |
| NC_000014.9 | FBJ murine osteosarcoma viral oncogene homolog |
| NC_000012.12 | Inositol 1,4,5-trisphosphate receptor, type 2 |
| NC_000014.7 | Uncharacterized LOC440200 |
| NC_000016.10 | Copine VII |
| NC_000019.10 | Cartilage intermediate layer protein 2 |
| AK094436.1 | Uncharacterized LOC284219 |
| NC_000022.11 | POM121 transmembrane nucleoporin-like 9, pseudogene |
| NCAS0811.11 | ASAP1 intronic transcript 1 |
| NC_000013.11 | Mitochondrial inner membrane organizing system 1 pseudogene 1 |
| NC_000002.12 | Ankyrin repeat domain 36B |
| NC_000022.11 | Galactose-3-O-sulfotransferase 1 |
| NC_000017.11 | RAS, dexamethasone-induced 1 |
| NC_000001.11 | Endothelin 2 |

Table 4. Major over or underrepresented GO classes during chondrogenesis

| Reporter ID | Reporter name | p value |
|-------------|---------------|---------|
| **Upregulated** | | |
| GO:0008768 | UDP-sugar diphosphatase activity | 0.001193246 |
| GO:0047631 | ADP-ribose diphosphatase activity | 0.002623259 |
| GO:0009046 | Zinc D-Ala-D-Ala carboxypeptidase activity | 0.004407889 |
| GO:0061473 | Murein tripeptide carboxypeptidase activity | 0.004407889 |
| GO:0005515 | Protein binding | 0.004645598 |
| GO:0008317 | Gurken receptor binding | 0.005477128 |
| GO:0004180 | Carboxypeptidase activity | 0.00784908 |
| GO:0004118 | Epidermal growth factor receptor binding | 0.008204381 |
| GO:0004114 | Cyclic-nucleotide phosphodiesterase activity | 0.008559554 |
| GO:0004112 | Cytochrome-c peroxidase activity | 0.00946968 |

| **Downregulated** | | |
| GO:0004118 | cGMP-stimulated cyclic-nucleotide phosphodiesterase activity | 0.000558946 |
| GO:0004120 | Photoreceptor cyclic-nucleotide phosphodiesterase activity | 0.000558946 |
| GO:0004119 | cGMP-inhibited cyclic-nucleotide phosphodiesterase activity | 0.000593312 |
| GO:0004117 | Calmodulin-dependent cyclic-nucleotide phosphodiesterase activity | 0.000628668 |
| GO:0004115 | 3′,5′-cyclic-AMP phosphodiesterase activity | 0.000740648 |
| GO:0047555 | 3′,5′-cyclic-GMP phosphodiesterase activity | 0.000740648 |
| GO:0004114 | 3′,5′-cyclic-nucleotide phosphodiesterase activity | 0.000861435 |
| GO:0004112 | Cyclic-nucleotide phosphodiesterase activity | 0.001539249 |
| GO:0004130 | Cytochrome-c peroxidase activity | 0.001946968 |

GO, gene ontology.
Table 5. Identification of genes differentially expressed during neurogenesis

| Reporter ID | Reporter name                                                                 | p value       |
|-------------|-------------------------------------------------------------------------------|---------------|
| **Upregulated** |                                                                               |               |
| GO:0008009  | Chemokine activity                                                            | 1.92E–08      |
| GO:0042379  | Chemokine receptor binding                                                     | 1.93E–08      |
| GO:0045236  | CXCR chemokine receptor binding                                                | 1.98E–08      |
| GO:0005125  | Cytokine activity                                                             | 1.02E–05      |
| GO:0031725  | CXCR6 chemokine receptor binding                                               | 1.24E–05      |
| GO:0005153  | Interleukin-8 receptor binding                                                 | 1.36E–05      |
| GO:0031723  | CXCR4 chemokine receptor binding                                               | 1.36E–05      |
| GO:0031724  | CXCR5 chemokine receptor binding                                               | 2.82E–05      |
| GO:0031732  | CCR7 chemokine receptor binding                                                | 2.94E–05      |
| **Downregulated** |                                                                           |               |
| GO:0010314  | Phosphatidylinositol-5-phosphate binding                                        | 0.001318916   |
| GO:0001010  | Sequence-specific DNA binding transcription factor recruiting transcription factor activity | 0.002113322  |
| GO:0010111  | Sequence-specific DNA binding RNA polymerase recruiting transcription factor activity | 0.002113322  |
| GO:0001034  | Sequence-specific DNA binding RNA polymerase III transcription factor activity  | 0.002113322  |
| GO:0001073  | DNA binding transcription antitermination factor activity                      | 0.002113322  |
| GO:0001130  | Sequence-specific DNA binding bacterial-type RNA polymerase transcription factor activity | 0.002113322  |
| GO:0001142  | Sequence-specific DNA binding mitochondrial RNA polymerase transcription factor activity | 0.002113322  |
| GO:0001167  | Sequence-specific DNA binding RNA polymerase I transcription factor activity    | 0.002113322  |
| GO:0001199  | Metal ion regulated sequence-specific DNA binding transcription factor activity  | 0.002113322  |

Table 6. Identification of genes differentially expressed during osteogenesis

| Reporter ID | Reporter name                                                                 | p value       |
|-------------|-------------------------------------------------------------------------------|---------------|
| **Upregulated** |                                                                               |               |
| GO:0001758  | Retinal dehydrogenase activity                                                | 3.27305E–06   |
| GO:0016620  | Oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor | 2.55861E–05   |
| GO:0003942  | N-acetyl-gamma-glutamyl-phosphate reductase activity                          | 0.000203654   |
| GO:0004030  | Aldehyde dehydrogenase [NAD(P)+] activity                                     | 0.000203654   |
| GO:0004043  | L-aminoacidopate-semialdehyde dehydrogenase activity                          | 0.000203654   |
| GO:0004073  | Aspartate-semialdehyde dehydrogenase activity                                 | 0.000203654   |
| GO:0008774  | Acetaldehyde dehydrogenase (acytlyating) activity                             | 0.000203654   |
| GO:0008863  | Formate dehydrogenase (NAD+) activity                                         | 0.000203654   |
| GO:0008883  | Glutamy-tRNA reductase activity                                               | 0.000203654   |
| GO:0008886  | Glyceraldehyde-3-phosphate dehydrogenase (NADP+) (nonphosphorylating) activity | 0.000203654   |
| **Downregulated** |                                                                       |               |
| GO:0004551  | Nucleotide diphosphatase activity                                             | 0.000113085   |
| GO:0016462  | Pyrophosphatase activity                                                      | 0.001860999   |
| GO:0030229  | Very low-density lipoprotein particle receptor activity                       | 0.003572689   |
| GO:0043183  | Vascular endothelial growth factor receptor 1 binding                         | 0.003572689   |
| GO:0043185  | Vascular endothelial growth factor receptor 3 binding                         | 0.003572689   |
| GO:0030021  | Extracellular matrix structural constituent conferring compression resistance  | 0.004052636   |
| GO:0030020  | Extracellular matrix structural constituent conferring tensile strength       | 0.004196858   |
| GO:0030197  | Extracellular matrix constituent, lubricant activity                          | 0.004196858   |
| GO:0030023  | Extracellular matrix constituent conferring elasticity                         | 0.004269826   |
| GO:0005539  | Glycosaminoglycan binding                                                     | 0.004273952   |
tion factors, including EBF1, Sp1, and E2F1. In particular, mutation of the E2F1-binding site obviously impairs the activity of the ATF5 promoter, showing that this site is the principal cis-element for the transcriptional activation of human ATF5 gene [12]. These facts could support our results showing that E2F1 was expressed highly during induction for osteogenic differentiation. IGFBP coordinate and regulate biological activities of IGF, a powerful neurotrophic factor that provokes proliferation, migration, and differentiation of glial and neuronal cells and prevents these cells from going through apoptosis [16]. Some authors have proposed that MSCs might have neurotrophic properties to differentiate into neuron and repair nerve. A previous study using a nerve injury model has shown a considerable increment in the release of IGFBP-1 from transplanted MSCs and IGFBP-1 during repair of peripheral nerves has significant influence on engraftment and neural differentiation of transplanted cells. Therefore, it has been suggested that IGFBP-1 is an essential neurotrophic factor released from MSCs that can promote regeneration of damaged neural tissues [17]. NGFRs are a group of growth factor receptors that can specifically bind to neurotrophins. They are also used as cell surface markers to define MSC phenotype [18]. In particular, in several studies using monoclonal antibodies to NGFR for isolation of NGFR positive MSCs, this subset of cells has a higher neuronal differentiation property than the whole MSCs population [19, 20]. These results were similar to our results showing that IGFBP-1 and NGFR were expressed highly during induction for neurogenic differentiation.

GO is a method to comprehend massive-scale gene expression data. In the GO database, top-level ontologies are molecular function, biological process, and cellular component [21]. In our study, according to GO analysis, GO terms related to molecular functions were overrepresented in all trilineage differentiation of hNTSCs. However, GO terms related to cytokine or chemokine were overrepresented in neural differentiated hNTSCs while GO terms related to catalysis and GO terms related to oxidoreductase activity were mainly overrepresented in chondrogenic and osteogenic differentiated hNTSCs, respectively. These facts clearly showed that differently differentiated MSCs had other specific characteristics. In the future, further studies are needed to find the meaning of other major overrepresented GO terms in differentiated hNTSCs.

We performed microarray analysis during trilineage differentiation of hNTSCs in vitro. We found that over 300 genes were expressed differentially in hNTSCs during differentiation. However, certain limitations need to be noted in this study. Of over 300 genes in hNTSCs we found, we could not evaluate characteristics of the majority of genes regulated during in vitro differentiation. Additionally studies are needed to determine changes of most genes detected by microarray analysis and characterize these genes more precisely. Additionally, it would be important to identify sequential gene expression during differentiation. Previously, temporal changes of cell growth and osteoblast phenotype-associated genes in human and rodent models have been reported [22, 23]. In future, we will evaluate sequential expression profiling of hNTSCs during differentiation. However, gene expression profiling and GO analysis of this study are useful for defining genes responsible for characteristics of hNTSCs and for analysis of responses of hNTSCs to signals that drive different differentiation processes. Our results may provide novel information of genes involved in the differentiation of hNTSCs.

Conclusions

We described genome-wide gene expression patterns of hNTSCs during trilineage differentiation. Differences in expression profiles of trilineage differentiated hNTSCs were found and 316 candidate genes were shown to be definitely up- or downregulated. Further analytical studies should be conducted to comprehensively determine functional roles of these differentially expressed genes detected by microarray analysis. In the future, gene expression data of hNTSCs could offer important understanding for cellular differentiation processes in MSCs.

Statement of Ethics

This study was approved by the institutional review board committee (IRB No. KC08TISS0341), and informed consent was obtained from all patients.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Sun Hwa Park: data analysis, drafting, final approval, and accountability for all aspects of the work; Do Hyun Kim: data analysis, drafting, final approval, and accountability for all aspects of the work; Mi Hyun Lim: data analysis, drafting, final approval, and accountability for all aspects of the work; Byeong Gon Yun: data analysis, drafting, final approval, and accountability for all aspects of the work; Jung Ho Jeun: data analysis, drafting, final approval, and accountability for all aspects of the work; Se Hwan Hwang: data analysis, drafting, final approval, and accountability for all aspects of the work; Sung Won Kim: data analysis, drafting, final approval, and accountability for all aspects of the work.

References

1 Hwang SH, Kim SY, Park SH, Choi MY, Kang HW, Seol YJ, et al. Human inferior turbinate: an alternative tissue source of multipotent mesenchymal stromal cells. Otolarngol Head Neck Surg. 2012 Sep;147(3):568–74.
2 Berger G, Balum-Azim M, Ophir D. The normal inferior turbinate: histomorphometric analysis and clinical implications. Laryngoscope. 2003 Jul;113(7):1192–8.
3 Hwang SH, Kim SY, Park SH, Choi MY, Back SA, Kim YI, et al. Osteogenic differentiation of human turbinate mesenchymal stromal cells. J Tissue Eng Regen Med. 2011 Dec;6(6):544–53.
4 Hwang SH, Kim SW, Kim SY, Park SH, Lee HJ, Choi MY, et al. Human turbinate mesenchymal stromal cells as a potential option for cartilage tissue engineering. J Tissue Eng Regen Med. 2011 Dec;6(6):536–43.
5 Mori G, Brunetti G, Oranger A, Carbone C, Ballini A, Lo Muzio L, et al. Dental pulp stem cells: osteogenetic differentiation and gene expression. Ann N Y Acad Sci. 2011 Nov;1237:47–52.
6 Aonuma H, Ogura N, Takahashi K, Fujimoto Y, Iwai S, Hashimoto H, et al. Characteristics and osteogenic differentiation of stem/progenitor cells in the human dental follicle analyzed by gene expression profiling. Cell Tissue Res. 2012 Nov;350(2):317–31.
7 Kim SH, Kim YS, Lee SY, Kim KH, Lee YM, Kim WK, et al. Gene expression profile in mesenchymal stem cells derived from dental tissues and bone marrow. J Periodontal Implant Sci. 2011 Aug;41(4):192–200.
8 Sharov AA, Piao Y, Ko MS. Gene expression profiling of mouse embryos with microarrays. Meth Enzymol. 2010;477:511–41.