## DATA NOTE

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# Time-course mass spectrometry data of adipose mesenchymal stem cells acquiring chondrogenic phenotype

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## Abstract

**Objectives:** Rabbit adipose mesenchymal stem cells were used for the purpose of studying acquisition of the chondrogenic phenotype over time at 1, 14 and 28 days after in vitro incubation with differentiation media, using nano-liquid chromatography electrospray ionization tandem mass spectrometry analysis. This was part of a preliminary study of the behavior of differentiated adipose stem cells for use in a rabbit model of laryngoplasty.

**Data description:** The data comprise .MGF, .RAW, .MZID, and .XLSX, lists of peaks, peptides and proteins identified by nano-flow liquid chromatography electrospray ionization tandem mass spectrometry analysis upon incubation with non-differentiating (ND) or chondrogenic differentiating (CHD) media (ProteomeXchange ID PXD010236). XLSX files contain the following information: day 1 CT (control, N = 3499 proteins), day 14 ND (N = 3106 proteins), day 28 ND (N = 3116 proteins), day 14 CHD (N = 2901 proteins), and day 28 CHD (N = 2876 proteins). Proteins are characterized with respect to their − 10lgP value, percent coverage, number of total as well as unique peptides after trypsin digestion, derivatization method (carbamidomethylation, oxidation, or combined carbamidomethylation + oxidation), average mass, and include a full description.

**Keywords:** Proteomics, Mass spectrometry, Adipose mesenchymal stem cells, Regenerative medicine, Chondrogenic differentiation time-course

## Objective

Adipose mesenchymal stem cells (ADSCs) are fast gaining popularity for various regenerative medicine applications due to their pluripotency as well as relative use of collection and processing. This has called for increased scrutiny and standardization of protocols involved in tissue collection, differentiation and analysis [1]. While analysis based on DNA or RNA expression has been the preferred method for studying gene expression due to the relatively low cost and flexible, scalable features of microarrays, these analyses do not report actual protein levels. Meanwhile, the cost of protein analysis using nano-flow liquid chromatography electrospray ionization tandem mass spectrometry (nLC–MS/MS) now allows for true protein measurements to be made at the tissue or even single cell level. Our lab's interest lies in developing regenerative medicine application for otorhinolaryngology. Currently, we are gaining expertise with new biologicals to address vocal fold (VF) paralysis, one of the complications that can arise from accidental sectioning of the recurrent laryngeal nerve (RLN) during thyroidectomy. As part of developing a rabbit model for injection medialization laryngoplasty (IML), a surgical procedure involving injection of volume augmenting materials that bring the paralyzed VF proximal to its counterpart, we established protocols for growing and differentiating ADSCs into chondrocytes in vitro. The data now allow for time-course analyses of true protein levels with the potential of revealing new proteins and protein network

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models implicated in the chondrogenic differentiation of ADSCs in vitro.

## Data description

### Data description

Five lists of proteins were generated by nLC–MS/MS analysis of rabbit ADSC incubated with non-differentiating (ND) or chondrogenic differentiating (CHD) media for 1, 14, or 28 days. List 1: day 1 (N = 3499 proteins); List 2: ND media, day 14 (N = 3106 proteins); List 3: ND media, day 28 (N = 3116 proteins); List 4: CHD media, day 14 (N = 2901 proteins); and List 5: CHD media, day 28 (N = 2876 proteins). Proteins are displayed in rows and descriptors in columns, as follows: (1) Accession Number, (2) Protein Group, (3) Protein ID, (4) − 10 logP value, (5) % Coverage, (6) No. of Total Peptides, (7) No. of Unique Peptides, (8) Derivatization Method, (9) Average Mass, and (10) Description. A Protein Group is a list of proteins sharing a significant number of identical matched peptides. − 10 logP expresses the confidence of making a protein call; high values reflect high confidence [2]. Coverage refers to the percentage of amino acids in the matched peptides relative to the total number of amino acids in the protein.

### Chondrogenic differentiation

Chondrogenic differentiation was performed as previously described [3]. Briefly, ADSCs were prepared in 15 mL polystyrene tubes as collagen macrospheres by mixing $5 \times 10^5$ cells with 50 μL of collagen followed by sedimentation at 500 g for 5 min and drop-wise addition of StemPro Chondrogenic differentiation media (Basal Medium + Chondrogenic Supplement, A10071-01 Life Technologies, Carlsbad, CA). This was supplemented with 5 ng/mL of TGF-β2 (T2815; Sigma-Aldrich, St. Louis, MO). Non-differentiating media consisted of the Basal Medium alone. All media was replenished every 2–3 days. Chondrogenic differentiation was confirmed by Alcian blue staining and sulfated glycosaminoglycan (s-GAG) level analysis with DNA content correction using PicoGreen (Quant-iT P7589, Invitrogen, Carlsbad, CA). Parallel cultures in adipogenic and osteogenic differentiation media (StemPro A1007001 and A1007201; ThermoFisher, Minneapolis, MN) were performed to

## Table 1  Overview of data files/data sets

| Label | Name of data set | File types (file extension) | Data repository and identifier (DOI or accession number) |
|---|---|---|---|
| File 1 | Day 1 Ctrl | MS/MS spectra file (.mgf) | ftp://massive.ucsd.edu/MSV000082505/peak/day1ctrl.mgf |
| File 2 | Day 1 Ctrl | Mass spectrometry data (.raw) | ftp://massive.ucsd.edu/MSV000082505/raw/day1ctrl.raw |
| File 3 | Day 1 Ctrl | Peptide data (.mzid) | ftp://massive.ucsd.edu/MSV000082505/result/day1ctrl_peptides_1_1_0.mzid |
| File 4 | ND day 14 | MS/MS spectra file (.mgf) | ftp://massive.ucsd.edu/MSV000082505/peak/day14ctrl.mgf |
| File 5 | ND day 14 | Mass spectrometry data (.raw) | ftp://massive.ucsd.edu/MSV000082505/raw/day14ctrl.raw |
| File 6 | ND day 14 | Peptide data (.mzid) | ftp://massive.ucsd.edu/MSV000082505/result/day14ctrl_peptides_1_1_0.mzid |
| File 7 | ND day 28 | MS/MS spectra file (.mgf) | ftp://massive.ucsd.edu/MSV000082505/peak/day28ctrl.mgf |
| File 8 | ND day 28 | Mass spectrometry data (.raw) | ftp://massive.ucsd.edu/MSV000082505/raw/day28ctrl.raw |
| File 9 | ND day 28 | Peptide data (.mzid) | ftp://massive.ucsd.edu/MSV000082505/result/day28ctrl_peptides_1_1_0.mzid |
| File 10 | CHD day 14 | MS/MS spectra file (.mgf) | ftp://massive.ucsd.edu/MSV000082505/peak/day14ctrl.mgf |
| File 11 | CHD day 14 | Mass spectrometry data (.raw) | ftp://massive.ucsd.edu/MSV000082505/raw/day14chond.raw |
| File 12 | CHD day 14 | Peptide data (.mzid) | ftp://massive.ucsd.edu/MSV000082505/result/day14chond_peptides_1_1_0.mzid |
| File 13 | CHD day 28 | MS/MS spectra file (.mgf) | ftp://massive.ucsd.edu/MSV000082505/peak/day28chond.mgf |
| File 14 | CHD day 28 | Mass spectrometry data (.raw) | ftp://massive.ucsd.edu/MSV000082505/raw/day28chond.raw |
| File 15 | CHD day 28 | Peptide data (.mzid) | ftp://massive.ucsd.edu/MSV000082505/result/day28chond_peptides_1_1_0.mzid |
| File 16 | Day 1 Ctrl | Protein list file (.xlsx) | ftp://massive.ucsd.edu/MSV000082505/updates/2018-07-19_ssanmarina1_eec23ce3/other/Day1_ctrl_proteins.xlsx |
| File 17 | ND day 14 | Protein list file (.xlsx) | ftp://massive.ucsd.edu/MSV000082505/updates/2018-07-19_ssanmarina1_eec23ce3/other/Day14_ctrl_proteins.xlsx |
| File 18 | CHD day 14 | Protein list file (.xlsx) | ftp://massive.ucsd.edu/MSV000082505/updates/2018-07-19_ssanmarina1_eec23ce3/other/Day14_chondro_proteins.xlsx |
| File 19 | ND day 28 | Protein list file (.xlsx) | ftp://massive.ucsd.edu/MSV000082505/updates/2018-07-19_ssanmarina1_eec23ce3/other/Day28_ctrl_proteins.xlsx |
| File 20 | CHD day 28 | Protein list file (.xlsx) | ftp://massive.ucsd.edu/MSV000082505/updates/2018-07-19_ssanmarina1_eec23ce3/other/Day28_chondro_proteins.xlsx |
| File 21 | Methods | Methods file (.docx) | ftp://massive.ucsd.edu/MSV000082505/methods/ProteomeXchange%20project%20description_final.docx |

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confirm the presence of pluripotent stem cells in our samples. These data were published elsewhere [3].

## nLC–MS/MS analysis

After 1, 14, or 28 days the cell pellets were washed ten times with PBS, fixed in 4% paraformaldehyde and processed for nLc–MS/MS analysis as previously reported [4]. The sample preparation protocol is available on line (Table 1, File 21: Methods). Tandem MS/MS peptide spectra were extracted using msConvert software (ProteoWizard) and analyzed with Mascot (Matrix Science), and X! tandem in order to match mass spectra to peptide sequences. N-terminus ammonia loses (Glu $\rightarrow$ pyro-Glu and gln $\rightarrow$ pyro-Glu), oxidation of methionine and the iodoacetamide derivative of cysteine were specified as variable modifications A fragment ion mass tolerance of 0.020 Da and parent ion tolerance of 10.0 PPM were specified in Mascot and X! Tandem. Scaffold (version 4.6.2, from Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and proteins. The Rabbit RefSeq database (12/2016 with 77,162 entries) was searched and hits verified against a decoy database. Peptide IDs were accepted if they could be established at greater than 95% probability by the Scaffold Local FDR algorithm. Protein IDs were accepted if they could be established at greater than 95% probability using Protein Prophet [5]. Only proteins containing two or more unique peptides were compared across groups [6].

## Limitations

The data presented here describe acquisition of the chondrogenic phenotype by rabbit ADSCs. The data are useful for the appreciation of time-dependent cellular changes in ADSCs at 14 and 28 days of incubation with chondrogenic differentiation media. The data are also useful for a comparison across species, for example with mouse or human ADSCs, aimed at identifying patterns of similar or differential expression of proteins implicate in acquisition of the chondrogenic phenotype. The main limitations of the data are the lack of replicate measurements and time points beyond day 28. Furthermore, because of the lack of a finer temporal resolution, transient, or intermediary events are not properly captured.

## Abbreviations

ADSC: adipose mesenchymal stem cells; CHD: chondrogenic differentiating (media); IML: injection medialization laryngoplasty; ND: non-differentiating (media); nLc–MS/MS: nano-liquid chromatography electrospray ionization tandem mass spectrometry; RLN: recurrent laryngeal nerve; s-GAG: sulfated glycosaminoglycan; VF: vocal fold.

## Authors' contributions

JRJ—study design/supervision, manuscript write-up/edit. SGV—lab experiments, data acquisition. BJM and MCC—data acquisition & processing. MSO

and DK—study consulting, experimental planning. SSM—data analysis, manuscript write-up. All authors read and approved the final manuscript.

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## Competing interests

The authors declare that they have no competing interests.

## Availability of data materials

Data can be freely and openly accessed at ProteomeXchange (http://www.identifiers.org/px/PXD010236). Please see Table 1 and reference list for details and links to the data.

## Consent for publication

Not applicable.

## Ethics approval and consent to participate

All animal experiments were performed in agreement with the Guide for the Care and Use of Laboratory Animals and the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, Mayo Clinic Institutional Animal Care and Use Committee (IACUC) Approval A52714.

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