Neural stem cells (NSCs) and neural progenitor cells (NPCs) are self-renewing and multipotent cells, however, NPCs are considered to be more lineage-restricted with a reduced self-renewing capacity. We present data comparing the expression of 21 markers encompassing pluripotency, self-renewal (NSC) as well as neuronal and glial (astrocyte and oligodendrocyte) lineage specification and 28 extracellular proteoglycan (PG) genes and their regulatory enzymes between embryonic stem cell (ESC)-derived human NSCs (hNSC H9 cells, Thermo Fisher) and human cortex-derived normal human NPCs (nhNPCs, Lonza). The data demonstrates expression differences of multiple lineage and proteoglycan-associated genes between hNSC H9 cells and nhNPCs. Data interpretation of markers and proteoglycans defining NSC and neural cell lineage characterisation can be found in “Cell surface heparan sulfate proteoglycans as novel markers of human neural stem cell fate determination” (Oikari et al. 2015) [1].

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DOI of original article: http://dx.doi.org/10.1016/j.scr.2015.12.011
* Correspondence to: Genomics Research Centre, Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, 60 Musk Ave, Kelvin Grove, QLD 4059, Australia.

E-mail address: larisa.haupt@qut.edu.au (L.M. Haupt).

http://dx.doi.org/10.1016/j.dib.2016.02.030
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### Specifications Table

| Subject area       | Cell biology                                                                 |
|--------------------|------------------------------------------------------------------------------|
| More specific sub-area | Human neural stem cell (hNSC) and human neural progenitor cell (hNPC)          |
| Type of data       | Text file, graphs and immunofluorescence images                               |
| How data was acquired | in vitro culture/expansion and phase-contrast fluorescence microscopy data for phenotypic analysis was obtained on an Olympus IX81 inverted fluorescent microscope via Volocity Imaging package; raw Q-PCR data was obtained on Applied Biosystems 7900HT Fast Real-Time PCR system |
| Data format        | Analysed                                                                     |
| Experimental factors | hNSC H9 and nhNP cells were cultured under basal medium conditions           |
| Experimental features | hNSC H9 cells (Thermo Fisher) were cultured as a monolayer and nhNP cells (Lonza) were cultured as neurospheres in standard maintenance medium provided by the manufacturer. RNA was harvested and transcribed to cDNA and gene expression of a panel of 49 genes examined by Q-PCR. Specific neural cell lineage markers were further detected through immunofluorescence (IF) |
| Data source location | Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland Australia |
| Data accessibility | Data is provided in this article                                             |

- The data provides an extensive panel of markers for better characterisation of human NSCs and NPCs.
- The data demonstrates significant and specific differences in expression of pluripotency, NSC self-renewal and neural cell lineage markers between hNSCs and hNPCs.
- The marker profile data could be used to identify and differentiate between the two cell types to improve their efficacy in research or therapeutic applications.
- The data provides information on the proteoglycan profile of human NSCs and NPCs providing potential new additional markers defining lineage progression of NSCs to NPCs.

### 1. Data

We compared the expression of 49 selected genes between human NSCs (hESC-derived hNSC H9 cells, Thermo Fisher) and normal human progenitor cells (nhNPCs, Lonza) following short-term culture under basal growth conditions. Q-PCR data was obtained for pluripotency genes, NSC, neuronal, astrocyte and oligodendrocyte lineage defining genes \((n = 21); \text{Table 1.}) (Fig. 1) with several of these markers also detected through immunofluorescence (IF) (Fig. 2) using specific antibodies (Table 3). In addition, Q-PCR data was obtained for 28 heparan and chondroitin sulphate proteoglycan biosynthesis enzymes and core protein genes (Table 2) ubiquitous to the neural niche [1–7] in hNSC H9 cells and nhNPCs (Figs. 3 and 4). The data presented provides information on self-renewal and multilineage potential as well as proteoglycan expression differences between the two neural stem/progenitor cell types.

### 2. Experimental design, materials and methods

#### 2.1. Cell culture

Gibco® human neural stem cells derived from NIH-approved H9 (WA09) embryonic stem cells (hNSC H9 cells) were cultured as a monolayer on Geltrex® coated culture dishes in StemPro® NSC serum-free medium (NSC SFM) containing KnockOUT™ DMEM/F-12 supplemented with 2% StemPro® Neural Supplement, 20 ng/ml FGFb and EGF and 2 mM GlutaMAX™ (cells and culture reagents obtained from Thermo
| Gene                        | Symbol   | Forward primer       | Reverse primer       | RefSeq          | Ref     |
|-----------------------------|----------|----------------------|----------------------|-----------------|---------|
| CD44                        | CD44     | AGCAACTGAGACAGCAACCA | AGACGTACACCCATTGTGT | NM_000610.3     |         |
| Doublecortin                | Dcx      | TATGCGCGCAAGCAAGTCTC | TACAGGTCTTGCTTCCGG  | NM_178152.2     |         |
| Enolase 2                   | ENO2     | TGCACAGGGCAAGTACAGAC | ACAGCAACTGAGATTACAG | NM_001975.2     |         |
| Forkhead box A2             | FOXA2    | CTGTGCCTTGTTTGTCCTTG | GGAGGAGTACCCCTCGG  | NM_021784.4     |         |
| Galactosylceramidase        | GalC     | GCCAAGGTATACATGATT  | TTTCACCTGGAGACCTTT  | NM_00120402.1   | [8]     |
| Glial fibrillary acidic protein | GFAP    | AGGGTGAGAGGGCAATGTG  | GTGGCTCTCTGCTCTTGCT | NM_002055.4     |         |
| Human telomerase            | hTERT    | GACGTGGAAGATGAGCGTG  | GACGACGTACACACTAC  | NM_00193376.1   | [9]     |
| Microtubule associated protein 2 | MAP2    | GACTGCAAGCTGCCTTCTAG | AAGTAAATCTCTTACCTGTGAC | NM_02374.3     |         |
| Mushahi I                   | MSI 1    | TGCAAGAGATCGCAGGGT  | CGATTGGCGACACATTAT | NM_002442.3     |         |
| Nanog homeobox              | NANOG    | AACTCTGACTAACACAGTTA | AAAGGCTGGGATTAGGT  | NM_024865.2     |         |
| Nestin                      | NES      | CTGAACTTCTGCCAACCTAA | GTGCTCAAGGGATACACG | NM_006617.1     |         |
| Neurofilament M             | NEFM     | TGACGTCAAGAGATCAGAG | GATGATCTGTAGTGAGCT | NM_005382.2     |         |
| Neurogenin 2                | NUMG2    | AGACGCAATAGATGGTCTA | CGATCCGAGAGCAGTAAAC | NM_024019.3     |         |
| Oligodendrocyte transcription factor 1 | OLIG1 | GATCCCTGGCTCCTACAGC | CTGCCAGCAGTAAGTTAG | NM_138983.2     | [8]     |
| Oligodendrocyte transcription factor 2 | OLIG2 | GACAGCTGGAGAGGGCAGG | CGCTTTGCTATCTTCTCT | NM_005806.3     | [8]     |
| POU Class 5 homeobox (OCT3/4) | OCT3/4 | ATCTCCAGATGCAAGACAA | TGATCTGCTGAGTGCTT | NM_002701.4     |         |
| SRY box 1                   | SOX1     | CAACAGGAGCGCCCTCAAC | CCTGCAAGATGCCTTCCAC | NM_005986.2     |         |
| SRY box 2                   | SOX2     | GCACCTGAAAGCTGCTTCTC | GGAGGAGAGGAGTACACAGG | NM_003106.3     | [10]    |
| S100 Calcium binding protein B | S100B | TTTCGAGAGGAGGGGACAG | CTCTGTCCTTCTGTTCTCT | NM_006272.2     |         |
| Vimentin                    | VIM      | GCACCAAGCTAACACAGCAA | GCATCAGCAAATCTTCTCT | NM_003380.3     |         |
| βIII tubulin                | TUBB3    | GCCAAGGTCTGGAAGTTCA  | CTGCAGGCAGTACTTGTGA | NM_008063.3     |         |
### Table 2
Primer sequences of proteoglycan associated genes.

| Gene                               | Symbol | Forward primer            | Reverse primer            | RefSeq        | Ref |
|------------------------------------|--------|---------------------------|---------------------------|--------------|-----|
| Aggrecan                           | AGG    | TGGCTACACCAAGCTACCT      | CGCTCAGCTCTTGAATGT        | NM_001135    |     |
| CS-Epimerase                       | CS-EP  | AGCTGTCAAGCCAACAAAAATAA  | CTACTACCAATCTAGCAGCAA     |              |     |
| Carbohydrate (chondroitin 6) sulfotransferase 3 | CHST3  | GGGTTTTTTGTAGTTGATTAGTTTCTT | GCTGGTCGCTGCTGTG          | NM_004273    |     |
| Carbohydrate (chondroitin 4) sulfotransferase 11 | CHST11 | CTGCTGGAAGTAGAGAGAGATGA  | GATGTTGCTCCAGGGAAGAG      | NM_018413    |     |
| Decorin                            | DCN    | TCTCTGACCCGCAT          | GAGTTCGCTCCAGGGAAGAG      | NM_001920.3  |     |
| Exostose 1                         | EXT1   | TGGACAGACAACACGAGATGA   | GCAAGCTTCTACGAATCTGAAG    | NM_000172.2  |     |
| Exostose 2                         | EXT2   | CAGCTAATTAAGGCCATCTCTTG | GGGATAGCAGGGAAGAG         | NM_000401    |     |
| Glypican 1                         | GC1    | GGACATCAAGCCAGCCGACAT   | TGTACTGGCCTCCTGCTG        |              |     |
| Glypican 2                         | GC2    | TGGATCAGCCACAGAGAGAGGT  | CAGCAGCAGGAGAGAGAGAGAGAG  |              |     |
| Glypican 3                         | GC3    | GGCATACCCGCAGCAAGAGAC   | GAGCTTCTCTCTCTCTGAGCAAG   |              |     |
| Glypican 4                         | GC4    | GGTGAATCCCGATACACCTCTACTTA | GCTCTTCGTCCTCAGCTATCAGT  |              |     |
| Glypican 5                         | GC5    | GCTACCTCTAGGCAACAAATAA  | GTCCTGAGCAGTCTCTCTACAGT   |              |     |
| Glypican 6                         | GC6    | CAGCTGCTTGAAGCAGTTCTT   | GATGTTGCTGCTGAGGATGT      |              |     |
| Heparanase                         | HPSE   | TCACACCCGAGCCAGAGAGAGAG | CTCTTGGACACCTGAGGATGT     |              |     |
| Heparan sulphate 2-O sulfotransferase 1 | HS2ST1 | CACGCTCAGCTAGCAAGGAGAGAAG | CAGGGGCACTCAGGAGATGAG   |              |     |
| Heparan sulphate 6-O sulfotransferase 1 | HS6ST1 | AGGGGCACTCAGCTAGCAAGGAGAGAAG | CAGGGGCACTCAGGAGATGAG   |              |     |
| Heparan sulphate 6-O sulfotransferase 2 | HS6ST2 | CTGGCTACCCGCTCTACTTCACTT | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| Heparan sulphate 6-O sulfotransferase 3 | HS6ST3 | ACATACCCGCTCTACTTCACTT | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| N-deacetylase/N-sulfotransferase 1 | NDS1   | TGGATCAGCAGGAGAGAGAGAGG  | CAGGGGCACTCAGGAGATGAG     |              |     |
| N-deacetylase/N-sulfotransferase 2 | NDS2   | CACGCTCAGCTAGCAAGGAGATGAG | CAGGGGCACTCAGGAGATGAG     |              |     |
| N-deacetylase/N-sulfotransferase 3 | NDS3   | ACATACCCGCTCTACTACTCTC  | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| N-deacetylase/N-sulfotransferase 4 | NDS4   | ATAGTATATATCTAGACAGGTGTACC | CAGGAAGCAGCAGAGAGAGATGAG |              |     |
| Perlecan                           | PER    | TGAGGACACCTGGCTTCTGGT   | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| Syndecan 1                         | SDC1   | TGGGTTGGTTCAATTGAGTTATTG | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| Syndecan 2                         | SDC2   | CTTGCTGTTTGTTCTCTTTTCTT | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| Syndecan 3                         | SDC3   | CTTGCTGTTTGTTCTCTTTTCTT | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| Syndecan 4                         | SDC4   | CTTGCTGTTTGTTCTCTTTTCTT | CACGCTCAGCTAGCAAGGAGATGAG |              |     |
| Versican                           | VCN    | TGGAATGATGTTCTCCTGAGAA  | AAGGTCTGCTCTTTCTCACAAGAG |              |     |
Fig. 1. Q-PCR analysis of pluripotency, NSC self-renewal, neuronal and glial lineage marker genes in hNSC H9 cells and nhNPCs. Relative expression in hNSC H9 cells and nhNPCs of: (A) pluripotency markers; (B) NSC self-renewal markers; (C) neuronal lineage defining markers; and (D) glial lineage defining markers. Relative expression normalised to 18 S, error bars = SD, statistical significance: * p < 0.05, ** p < 0.01, *** p < 0.001.
Fig. 2. Phenotypic and IF examination of NSC and neural lineage markers in hNSC H9 cells and nhNPCs. Phase-contrast images (20× magnification with 10× magnification inset, scale bar 130 μM) of: (A) hNSC H9 cells at P3 and (B) nhNPCs attached to surface (CC2 chamber slide) during expansion. Immunofluorescence (20× magnification, scale bar 130 μM) in hNSC H9 P3 cells and nhNPCs of stemness and lineage markers: (C) NSC self-renewal markers Nestin (FITC/green) and SOX2 (Cy3/yellow); and (D) neuronal marker MAP2 (FITC/green), astrocyte marker GFAP (Cy3/yellow) and oligodendrocyte marker O1 (AF594/red).
Fisher). hNSC H9 cells were cultured in p35 (10 cm²) dishes with culture medium changed every two days and cells passaged at 90% confluence using TrypLE. hNSC H9 cells were passaged twice and harvested for RNA at passage 3 (P3). Normal Human Neural Progenitor Cells (nhNPCs) isolated from the human brain cortex were cultured as neurospheres in Neural Progenitor Maintenance BulletKit™ medium (NPMM) containing 200 mL of Neural Progenitor Basal Medium supplemented with 0.4 mL rhFGF-B, 0.4 mL rhEGF, 4 mL Neural Survival Factor-1 and 0.4 mL Gentamicin/Amphotericin (cells and culture reagents obtained from Lonza). nhNPC neurosphere cultures were established by defrosting the cell ampule according to the manufacturer’s instructions and dividing the cells into two T75 (75 cm²) flasks containing 20 mL of NPMM. hNSC H9 and nhNPCs cultures were maintained in 5% CO₂ at 37 °C humidified atmosphere with phenotype of the cells monitored under an Olympus IX81 inverted phase-contrast microscope.

2.2. RNA extraction

RNA was harvested from cultured cells using TRIzol™ reagent (Invitrogen) using the Direct-zol™ RNA miniprep kit (Zymo Research) according to the manufacturer’s instructions with samples treated in-column with DNase I (Zymo Research). RNA was eluted in RNase-free H₂O and concentration and quality of RNA determined with a NanoDrop spectrophotometer (Thermo Scientific).

2.3. cDNA synthesis

For conversion of RNA into cDNA, 150 ng of RNA was incubated with 200 ng of Random Primer (New England BioLabs) at 65 °C for 10 min in a reaction made up to 20 µl with Milli-Q-H₂O. Samples were then incubated with 10 U of Transcriptor Reverse Transcriptase (Roche) and 1 mM dNTPs (New England Biolabs), 20 U of RNaseOUT (Invitrogen) in 1x RT reaction buffer in a total reaction volume of 30 µl. For the reverse transcription reaction samples were incubated at 25 °C for 10 min, then at 55 °C for 30 min and finally at 85 °C for 5 min. Concentration and quality of cDNA was measured on a NanoDrop spectrophotometer and cDNA was diluted to 40 ng/mL working concentrations.

2.4. Quantitative real-time PCR

Relative gene expression was detected using quantitative real-time PCR (Q-PCR). The 10 µl reaction volume contained 5 µl of SYBR®-Green PCR Master Mix (Promega), 200 ng of forward and reverse primer, 0.1 µl CXR reference dye (Promega) and 120 ng cDNA template. Amplification was monitored on an Applied Biosystems 7900HT Fast Real-Time PCR system with an enzyme activation of 2 min at 50 °C and 3 min at 95 °C followed by 50 cycles of 3 s at 95 °C and 30 s at 60 °C. The cycle
threshold (Ct) values were normalised against the endogenous control 18S (forward primer TTTCGAGGCCCTGTAATTGGA, reverse primer GCA GCAACTTAATATACGCTAT) Ct values (ΔCt value) included in each run, and relative gene expression was determined by the ΔΔCt value ($2^{−\Delta\DeltaCt}$). For ease of graphic presentation of relative gene expression, ΔΔCt values were multiplied by 10^6. Primer sequences for detected NSC and neural lineage genes are presented in Table 1 and primer sequences for heparan and chondroitin sulphate proteoglycan associated genes are presented in Table 2.

2.4.1. Immunofluorescence (IF)
Expression of selected NSC and neural lineage marker proteins were detected via IF using an Olympus IX81 inverted phase-contrast fluorescent microscope and images acquired using Volocity software (Perkin-Elmer) on a Hamamatsu Orca camera. For imaging, cells were plated on 8-well CC2-coated chamber slides (Lab-Tek) at 20–30 × 10^4 cells/well and cells were cultured for 3–4 days before fixing and staining. Briefly,
culture medium was removed, cells rinsed with 1× PBS with Ca\textsuperscript{2+} and Mg\textsuperscript{2+} and fixed with 4% paraformaldehyde. After this cells were blocked (5% Donkey serum, 1% BSA in PBS with or without 0.1% Triton-X to allow permeabilisation) and primary antibodies were incubated overnight at 4 °C. Isotype control antibodies were used as a negative control. After 24 h incubation, primary antibodies were removed, cells rinsed with 1× PBS with Ca\textsuperscript{2+} and Mg\textsuperscript{2+} and cells incubated with secondary antibodies for 2 h at room temperature. Finally, cells were rinsed with 1× PBS with Ca\textsuperscript{2+} and Mg\textsuperscript{2+} and slides mounted with DAPI (ab104139, Abcam). Antibodies and dilutions used are presented in Table 3.

2.4.2. Statistical analysis

For Q-PCR analysis each gene was detected in quadruplicate per sample. Paired t-test was used to determine statistical significance and defined as * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \). Error bars represent SD.
Acknowledgements

This work was supported through Queensland University of Technology postgraduate research awards (QUTPRA and IPRS) and the Estate of the late Clem Jones AO.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.02.030.

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