Data in Brief

Genome-wide copy number profiling to detect gene amplifications in neural progenitor cells

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DNA sequence amplification occurs at defined stages during normal development in amphibians and flies and seems to be restricted in humans to drug-resistant and tumor cells only. We used array-CGH to discover copy number changes including gene amplifications and deletions during differentiation of human neural progenitor cells. Here, we describe cell culture features, DNA extraction, and comparative genomic hybridization (CGH) analysis tailored towards the identification of genomic copy number changes. Further detailed analysis of amplified chromosome regions associated with this experiment, was published by Fischer and colleagues in PLOS One in 2012 (Fischer et al., 2012). We provide detailed information on deleted chromosome regions during differentiation and give an overview on copy number changes during differentiation induction for two representative chromosome regions.

Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30636.

Experimental design, materials and methods

Cell culture and differentiation

NHNP cells (P1) were grown in 75 cm² cell culture flasks with NPMM (neuronal progenitor maintenance medium) for initial 24 h after thawing. For the undifferentiated approach NHNP cells (approximately 4 × 10⁵ cells) were cultivated for additional 24 h in NPMM, harvested and the cell pellet was frozen before DNA extraction. For differentiation induction NHNP cells were transferred to 25 cm² laminin-coated cell culture flasks in NPDM (neural progenitor differentiation medium) supplemented with BDNF (brain-derived neurotrophic factor) at 25 ng/ml. We used approximately 4 × 10⁵ cells for 24 h differentiation induction, approximately 2.5 × 10⁵ cells for 48 h differentiation induction and approximately 2.5 × 10⁵ cells for 5d differentiation induction approach. Cells were harvested and cell pellet was frozen before proceeding to DNA extraction.

DNA extraction

Cell pellets were resuspended in lysis buffer (75 mM NaCl, 25 mM EDTA, pH 8) with 10% SDS. Undifferentiated NHNP cells, 24 h differentiation-induced and 5d differentiation-induced NHNP cell pellets were treated with proteinase K for >18 h at 55 °C. 48 h differentiation-induced NHNP cell pellets were treated with proteinase K for 5 h at 55 °C. All samples were extracted with 6 M NaCl/chloroform for 1 h on a rotator, centrifuged and the aqueous layer was precipitated with isopropanol and/or with sodium acetate ethanol. Genomic DNA from blood lymphocytes was extracted accordingly with proteinase K digest for >18 h at 55 °C. Genomic DNA from male and female healthy individuals was pooled.

Array-CGH data analysis

The array-CGH experiments were done with independently derived primary cells with different lot numbers. Array data were deposited in...
Table 1
Overview of deleted chromosome regions.
Start and end points of deleted chromosome regions are according to NCBI36/HG18.

| Deleted chromosomal regions in undifferentiated NHNP cells | Start | End | log2 | Size (Mb) |
|----------------------------------------------------------|-------|-----|------|-----------|
| chr1                                                     | 1962499 | 2837499 | −0.157 | 0.87 |
| chr2                                                     | 13312499 | 13712499 | −0.105 | 0.40 |
| chr3                                                     | 50137499 | 50562499 | −0.148 | 0.42 |
| chr4                                                     | 120387499 | 128912499 | −0.117 | 0.32 |

| Deleted chromosome regions in 1d differentiating NHNP cells | Start | End | log2 | Size (Mb) |
|-----------------------------------------------------------|-------|-----|------|-----------|
| chr1                                                     | 150737499 | 151562499 | −0.145 | 0.82 |
| chr2                                                     | 89037499 | 89887499 | −0.121 | 0.85 |
| chr3                                                     | 116887499 | 118062499 | −0.126 | 1.17 |
| chr4                                                     | 132212499 | 132762499 | −0.153 | 0.55 |

| Deleted chromosome regions in 2d differentiating NHNP cells | Start | End | log2 | Size (Mb) |
|-----------------------------------------------------------|-------|-----|------|-----------|
| chr1                                                     | 68912499 | 70687499 | −0.123 | 1.77 |
| chr2                                                     | 44062499 | 49762499 | −0.109 | 5.70 |
| chr3                                                     | 68912499 | 70687499 | −0.139 | 1.77 |
| chr4                                                     | 104487499 | 105012499 | −0.157 | 0.52 |

| Deleted chromosome regions in 5d differentiating NHNP cells | Start | End | log2 | Size (Mb) |
|-----------------------------------------------------------|-------|-----|------|-----------|
| chr1                                                     | 587212499 | 68587499 | −0.100 | 11.37 |
| chr2                                                     | 28237499 | 32287499 | −0.106 | 4.05 |
| chr3                                                     | 40637499 | 43612499 | −0.100 | 2.97 |
| chr4                                                     | 43862499 | 66987499 | −0.130 | 23.12 |

| Deleted chromosomal regions in undifferentiated NHNP cells | Start | End | log2 | Size (Mb) |
|----------------------------------------------------------|-------|-----|------|-----------|
| chr1                                                     | 138112499 | 139262499 | −0.178 | 1.15 |
| chr2                                                     | 130087499 | 135326317 | −0.114 | 5.23 |
| chr3                                                     | 123337499 | 123612499 | −0.166 | 0.27 |
| chr4                                                     | 112837499 | 114108681 | −0.103 | 1.72 |
| chr5                                                     | 75637499 | 75962499 | −0.132 | 0.32 |
| chr6                                                     | 83912499 | 84237499 | −0.212 | 0.32 |

| Deleted chromosome regions in 1d differentiating NHNP cells | Start | End | log2 | Size (Mb) |
|-----------------------------------------------------------|-------|-----|------|-----------|
| chr1                                                     | 150512499 | 150937499 | −0.267 | 0.42 |
| chr2                                                     | 89037499 | 89887499 | −0.121 | 0.85 |
| chr3                                                     | 116887499 | 118062499 | −0.126 | 1.17 |
| chr4                                                     | 132212499 | 132762499 | −0.153 | 0.55 |

| Deleted chromosome regions in 2d differentiating NHNP cells | Start | End | log2 | Size (Mb) |
|-----------------------------------------------------------|-------|-----|------|-----------|
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| chr2                                                     | 44062499 | 49762499 | −0.109 | 5.70 |
| chr3                                                     | 68912499 | 70687499 | −0.139 | 1.77 |
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GEO under accession number GSE30636. Signal intensity data were extracted from scanned images of each array using Roche NimbleGen NimbleScan v2.6 software. After spatial correction, the Cy3 and Cy5 signal intensities were normalized using qspline normalization. Following normalization a 10× window-averaging step is applied. For amplification and deletion detection we used the dynamic segMNT algorithm that identifies segments by minimizing the squared error relative to the segment means. To detect representative alterations and to minimize the identification of random alterations, we extracted segments with segment means greater 0.1 threshold and a size greater than 250 kb. Deletions detected in undifferentiated, 24 h differentiated, 48 h differentiated and 5 d-differentiated NHNP cells were summarized in Table 1.

The array plots at 25 kb resolution obtained by segmentation algorithm impressively demonstrate changes of the complex pattern of different copy numbers along a given chromosome. Fig. 1 summarizes the array plots for all probes of chromosome 12 and Fig. 2 of the array plots of all probes for chromosome 17. Interestingly at day zero the pattern of log2 ratios appears rather smooth. However, only after a 1 day-differentiation a wavy pattern appears that increases in number and amplitude heights over time. Recently, several studies explained the wavy CGH pattern by DNA extraction and replication timing [1,2].

Our results, however, do not support this hypothesis as we detected wavy CGH pattern indicative of imbalances in cells seeded for differentiation in different cell densities. In addition, DNA digestion with proteinase K for 5 h or >18 h did not lead to reduction of the wavy CGH pattern. In fact after 5d of differentiation and after more than 18 h protein digest we detected the highest amplitudes for copy number changes as shown in Figs. 1 and 2. Further gene amplification analysis using fluorescence in situ hybridization confirmed our results [3].
Discussion

Here, we report detailed information on DNA extraction method used for detection of copy number changes using NimbleGen 730K whole genome array. Here and in our previous report we detected a complex pattern of amplifications and deletions. This wavy pattern of copy number changes was independent from cell number and protein digest duration. This dataset is a first step towards uncovering copy number changes upon differentiation in human stem cells.

References

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