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Data in Brief

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

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ABSTRACT

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.

Specifications	
Organism/cell line/tissue	*Homo sapiens*
Sex	Male
Sequencer or array type	NimbleGen 720 K human whole genome tiling arrays.
Data format	Raw data: PAIR file, analyzed data: txt file
Experimental factors	NHNP cells vs normal mixed blood lymphocytes, NHNP cells at various time points of differentiation
Experimental features	NHNP cells were grown in Maintenance medium for 24 h. Differentiation was induced by withdrawal of EGF and FGF and addition of BDNF. Array-CGH experiments were done before differentiation, 24 h, 2d and 5d after differentiation induction.
Consent	n/a
Sample source location	NHNP cells form Lonza, Walkersville Inc. MD, USA

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 cm^2 cell culture flasks with NPMM (neuronal progenitor maintenance medium) for initial 24 h after thawing. For the undifferentiated approach NHNP cells (approximately 4×10^5 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 cm^2 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 4×10^5 cells for 24 h differentiation induction, approximately 2.5×10^5 cells for 48 h differentiation induction and approximately 2.5×10^5 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

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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					Deleted chromosome regions in 1d differentiating NHNP cells					Deleted chromosome regions in 2d differentiating NHNP cells					Deleted chromosome regions in 5d differentiating NHNP cells				
	Start	End	$\log_2$	Size (Mb)		Start	End	$\log_2$	Size (Mb)		Start	End	$\log_2$	Size (Mb)		Start	End	$\log_2$	Size (Mb)
										chr1	150737499	151562499	−0.145	0.82	chr1	150512499	150937499	−0.267	0.42
chr1	1962499	2837499	−0.157	0.87															
															chr2	89037499	89887499	−0.121	0.85
															chr2	91262499	94762499	−0.174	3.50
															chr2	116887499	118062499	−0.126	1.17
															chr2	132212499	132762499	−0.153	0.55
chr3	13312499	13712499	−0.105	0.40															
chr3	50137499	50562499	−0.148	0.42															
chr3	128587499	128912499	−0.117	0.32															
															chr4	69212499	70112499	−0.100	0.90
															chr5	22062499	30987499	−0.110	8.92
										chr5	26587499	27012499	−0.145	0.42					
															chr5	44062499	49762499	−0.109	5.70
					chr5	68912499	70687499	−0.123	1.77	chr5	68912499	70687499	−0.139	1.77	chr5	68937499	70687499	−0.188	1.75
															chr5	104487499	105012499	−0.157	0.52
chr5	177487499	177787499	−0.129	030															
															chr6	57212499	68587499	−0.100	11.37
															chr6	140462499	141187499	−0.102	0.72
										chr8	71787499	72112499	−0.105	0.32					
					chr8	15387499	15737499	−0.109	0.35										
															chr9	10337499	11612499	−0.109	1.27
															chr9	28237499	32287499	−0.106	4.05
															chr9	38787499	40412499	−0.150	1.62
															chr9	40637499	43612499	−0.100	2.97
															chr9	43862499	66987499	−0.130	23.12
										chr9	66987499	67262499	−0.182	0.27					
chr9	138112499	139262499	−0.178	1.15															
															chr10	8237499	9287499	−0.106	1.05
															chr10	55887499	56487499	−0.136	0.60
chr10	130087499	135326317	−0.114	5.23															
															chr11	48312499	49662499	−0.126	1.35
															chr11	49762499	56637499	−0.104	6.87
										chr12	28662499	28937499	−0.107	0.27					
															chr12	59437499	59762499	−0.146	0.32
															chr12	71462499	72062499	−0.153	0.60
chr12	123337499	123612499	−0.166	0.27															
															chr13	18337499	18612499	−0.116	0.27
															chr13	52987499	57062499	−0.107	4.07
chr13	112387499	114108681	−0.103	1.72															
					chr14	18137499	18412499	−0.100	0.27						chr14	18137499	18412499	−0.224	0.27
chr15	75637499	75962499	−0.132	0.32															
					chr16	1212499	1487499	−0.210	0.27										
															chr16	33687499	34312499	−0.128	0.62
										chr16	34637499	45137499	−0.114	10.5	chr16	34612499	35037499	−0.150	0.42
chr16	83912499	84237499	−0.212	0.32															
															chr17	21862499	22137499	−0.145	0.27
					chr18	13962499	14412499	−0.101	0.45										
					chr19	24087499	32887499	−0.172	8.80	chr19	24287499	33037499	−0.139	8.75					
										chr20	25837499	28087499	−0.139	2.25	chr20	25837499	29437499	−0.128	3.60

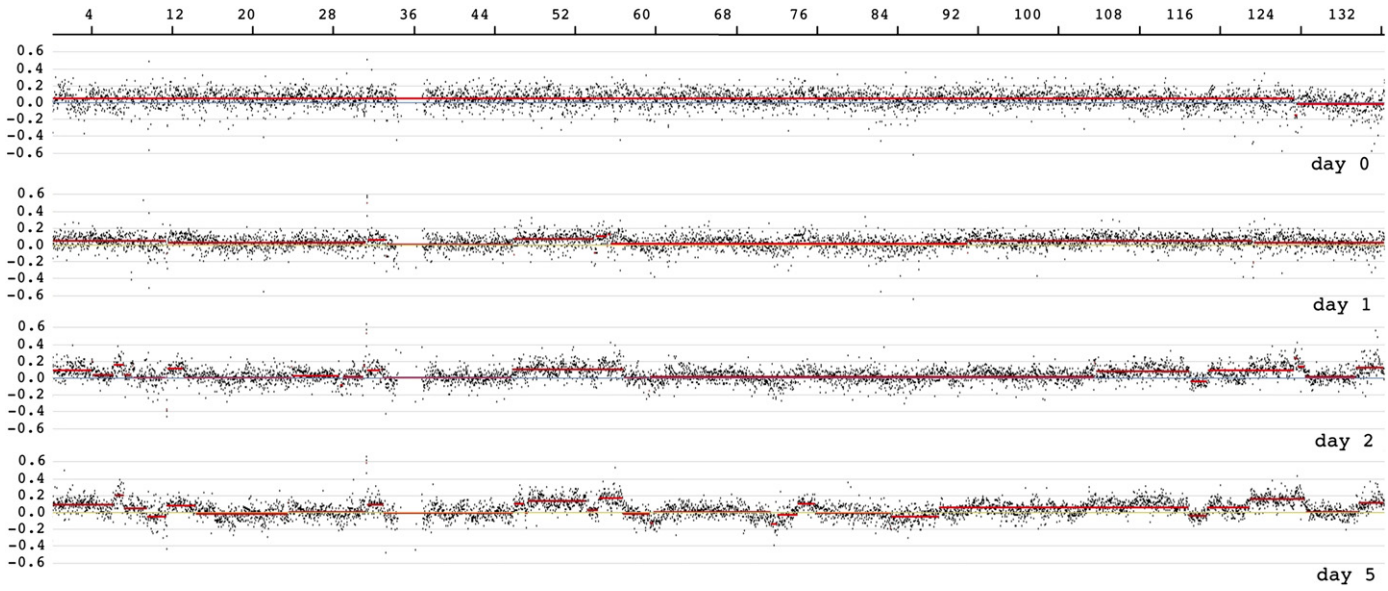

Fig. 1. Copy number changes of chromosome 12 after differentiation induction. Representative $\log_2$ ratio plots at 25 kb resolution for undifferentiated (0d), and for 1d, 2d and 5d differentiated NHNP cells were shown with base count on the x-axis and $\log_2$ ratio on the y-axis. Red bars depict extracted segments.

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 $\log_2$ 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].

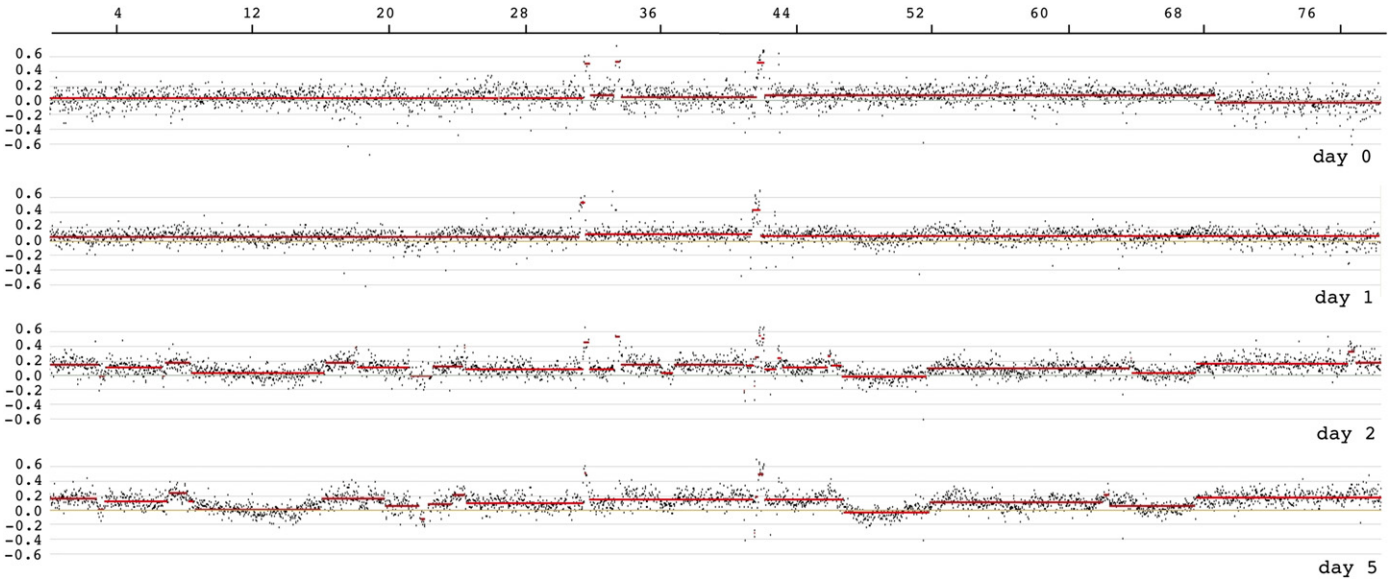

Fig. 2. Copy number changes of chromosome 17 after differentiation induction. Representative $\log_2$ ratio plots at 25 kb resolution for undifferentiated (0d), and for 1d, 2d and 5d differentiated NHNP cells were shown with base count on the x-axis and $\log_2$ ratio on the y-axis. Red bars depict extracted segments.

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.

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