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Data in Brief

Genome-wide copy number profiling of mouse neural stem cells during differentiation

U. Fischer [a,*], N. Ludwig [a], A. Keller [b], C. Backes [b], E. Meese [a]

[a] Department of Human Genetics, Saarland University, Building 60, 66421 Homburg/Saar, Germany
[b] Clinical Bioinformatics, Saarland University, Building E2.1, 66123 Saarbrücken, Germany

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ABSTRACT

There is growing evidence that gene amplifications were present in neural stem and progenitor cells during differentiation. We used array-CGH to discover copy number changes including gene amplifications and deletions during differentiation of mouse neural stem cells using TGF-ß and FCS for differentiation induction. Array data were deposited in GEO (Gene Expression Omnibus, NCBI) under accession number GSE35523. Here, we describe in detail the cell culture features and our TaqMan qPCR-experiments to validate the array-CGH analysis. Interpretation of array-CGH experiments regarding gene amplifications in mouse and further detailed analysis of amplified chromosome regions associated with these experiments were published by Fischer and colleagues in Oncotarget (Fischer et al., 2015). We provide additional information on deleted chromosome regions during differentiation and give an impressive overview on copy number changes during differentiation induction at a time line.

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Specifications	
Organism/cell line/tissue	*Mus musculus*
Sex	n.d.
Sequencer or array type	NimbleGen 720K mouse whole genome tiling arrays.
Data format	Raw data: PAIR file, analyzed data: txt file
Experimental factors	SFME cells vs normal mouse genomic DNA, SFME cells grown as spheres and after differentiation induction using TGF-ß or FCS
Experimental features	SFME cells were grown as spheres for undifferentiated state. Differentiation was induced by withdrawal of EGF and addition of TGF-ß or FCS. Array-CGH experiments were done with undifferentiated cells, 24 h-TGF-ß differentiation induced cells and 12 h-FCS differentiation induced cells.
Consent	n/a
Sample source location	SFME cells (CRL-9392™) from ATCC

1. Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35523.

2. Experimental design, materials and methods

2.1. Cell culture and differentiation

SFME cells cultured in the absence of fibronectin formed spheres and served as non-differentiated controls. SFME cells were seeded on fibronectin-coated cultureware and allowed to grow for 18 h prior to differentiation induction with TGF-β or FCS. SFME cells were differentiation induced using above supplemented ATCC DMEM:F12 Medium containing TGF-β (10 ng/ml) for 8 h, 12 h and 24 h or DMEM:F12 supplemented with FCS for 8 h, 12 h and 24 h.

Cells were harvested and cell pellet was frozen before proceeding to DNA extraction as described previously (Fischer et al., 2014 genomics data) [1].

2.2. Array-CGH data analysis

Array data were deposited in GEO under accession number GSE35523.

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

* Corresponding author.
E-mail address: ulrike.fischer@uks.eu (U. Fischer).

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Table 1
Overview of deleted chromosome regions.
Start and end points of deleted chromosome regions are according to NCBI37/mm9. Size is displayed in kb.

Sphere					24 h TGF-ß					12 h FCS				
	Start	End	log_2	Size		Start	End	log_2	Size		Start	End	log_2	Size
					chr1	3019999	9259999	−0.11308	6240					
					chr1	10419999	12539999	−0.16881	2120					
					chr1	21459999	33339999	−0.12453	11,880					
					chr1	47059999	51179999	−0.20953	4120					
					chr1	67859999	68979999	−0.20877	1120					
					chr1	95859999	106179999	−0.11556	10,320					
					chr1	108899999	120099999	−0.13136	11,200					
chr1	110459999	112459999	−0.15437	2000										
chr1	125099999	125779999	−0.1133	680										
					chr1	141739999	151499999	−0.12807	9760					
chr1	157499999	166019999	−0.11672	8520						chr1	157699999	164339999	−0.11251	6640
										chr1	179699999	180099999	−0.17032	400
					chr2	39299999	49379999	−0.11759	10,080					
					chr2	80899999	83139999	−0.16313	2240					
chr2	85619999	89979999	−0.1151	4360	chr2	85539999	89979999	−0.19832	4440					
chr2	94819999	101179999	−0.10144	6360	chr2	94419999	101179999	−0.14893	6760					
chr2	140259999	140739999	−0.10916	480										
chr2	174619999	176979999	−0.10819	2360	chr2	174539999	176979999	−0.10409	2440					
					chr3	3179999	7819999	−0.16459	4640					
chr3	10779999	15219999	−0.10217	4440	chr3	10699999	14179999	−0.21013	3480					
chr3	15259999	15819999	−0.25966	560	chr3	15339999	18379999	−0.18784	3040	chr3	15459999	15779999	−0.2969	320
					chr3	23219999	26019999	−0.12403	2800					
					chr3	41659999	48699999	−0.20394	7040					
chr3	47419999	48019999	−0.20803	600	chr3	48739999	50819999	−0.10307	2080					
					chr3	66659999	67219999	−0.14445	560					
					chr3	69859999	71299999	−0.14	1440					
chr3	71339999	73539999	−0.1502	2200	chr3	71339999	72859999	−0.24188	1520					
					chr3	72899999	75019999	−0.12964	2120					
					chr3	76179999	78579999	−0.10363	2400					
chr3	80059999	81099999	−0.14237	1040	chr3	80659999	81059999	−0.20763	400					
chr3	93699999	94059999	−0.18368	360	chr3	93699999	94059999	−0.16031	360					
					chr3	110219999	115099999	−0.16927	4880					
					chr3	116859999	120779999	−0.1109	3920					
chr3	123499999	125779999	−0.2668	2280	chr3	123339999	125779999	−0.35956	2440	chr3	123059999	127819999	−0.16916	4760
chr3	125819999	127739999	−0.16144	1920	chr3	125819999	128939999	−0.16294	3120					
					chr3	131419999	132379999	−0.13963	960					
					chr3	140139999	140899999	−0.18969	760					
					chr3	149859999	151299999	−0.18356	1440					
					chr3	154499999	159578619	−0.10621	5079					
					chr4	12379999	32099999	−0.13785	19,720					
					chr4	35779999	39859999	−0.20166	4080					
					chr4	64579999	75579999	−0.11545	11,000					
chr4	75659999	80779999	−0.2069	5120	chr4	75619999	80779999	−0.2917	5160	chr4	75579999	78339999	−0.19677	2760
					chr4	89259999	94339999	−0.13013	5080					
					chr5	5939999	7859999	−0.15729	1920					
					chr5	11859999	19539999	−0.12821	7680					
					chr5	54859999	61859999	−0.20136	7000					
					chr5	67699999	72699999	−0.13854	5000					
					chr5	78299999	91059999	−0.10839	12,760	chr5	81459999	81739999	−0.14919	280
										chr5	146259999	146579999	−0.10129	320
chr6	41499999	47339999	−0.10686	5840	chr6	41539999	47099999	−0.16256	5560					
					chr6	55739999	66419999	−0.10699	10,680					
					chr6	73379999	81459999	−0.11413	8080					
					chr6	103779999	112019999	−0.1065	8240					
chr6	138299999	140059999	−0.14334	1760	chr6	138379999	140059999	−0.22473	1680					
					chr7	56899999	70659999	−0.10699	13,760					
					chr7	75539999	79379999	−0.11891	3840					
					chr7	91779999	103339999	−0.10018	11,560					
chr7	10699999	12139999	−0.24888	1440										
chr7	110659999	111699999	−0.23809	1040	chr7	110619999	111379999	−0.20134	760					
					chr8	4579999	9659999	−0.11752	5080					
chr8	99539999	106019999	−0.10809	6480										
					chr8	29979999	34539999	−0.13338	4560					
					chr8	49459999	55619999	−0.16084	6160					
					chr8	98739999	106419999	−0.17182	7680					
					chr9	3139999	7299999	−0.10507	4160					
					chr9	10419999	12939999	−0.15382	2520					
					chr9	16739999	20259999	−0.10813	3520					
					chr9	33219999	34019999	−0.11795	800					
chr9	35659999	35939999	−0.21324	280	chr9	35659999	36299999	−0.16454	640	chr9	35659999	36059999	−0.16676	400
chr9	37699999	38899999	−0.10023	1200	chr9	37419999	39979999	−0.10149	2560					
										chr9	71699999	72019999	−0.1087	320

Table 1 (*continued*)

Sphere					24 h TGF-ß					12 h FCS				
	Start	End	$\log_2$	Size		Start	End	$\log_2$	Size		Start	End	$\log_2$	Size
					chr9	115059999	115619999	−0.10853	560					
					chr10	15059999	16899999	−0.1919	1840					
chr10	35579999	35939999	−0.17695	360	chr10	26739999	38059999	−0.11928	11,320					
chr10	45899999	51019999	−0.12617	5120	chr10	45899999	49499999	−0.20934	3600	chr10	47739999	49099999	−0.13582	1360
					chr10	63059999	65899999	−0.11266	2840					
					chr10	71099999	74299999	−0.10106	3200					
chr10	100819999	105099999	−0.16547	4280	chr10	100819999	105099999	−0.22175	4280	chr10	101059999	104739999	−0.11145	3680
					chr10	111619999	114339999	−0.19567	2720					
					chr10	122579999	126139999	−0.11643	3560					
chr10	128539999	129975647	−0.10464	1436	chr10	128539999	129975647	−0.14084	1436					
					chr11	8979999	18619999	−0.11164	9640	chr11	17579999	18619999	−0.10657	1040
chr11	36019999	42459999	−0.11313	6440	chr11	36459999	42459999	−0.19152	6000					
					chr11	90499999	93299999	−0.14477	2800					
					chr12	89939999	99619999	−0.12512	9680					
					chr12	114979999	116219999	−0.10697	1240					
					chr13	76539999	90659999	−0.12234	14,120					
					chr13	115939999	120282113	−0.12049	4342					
					chr14	49819999	53379999	−0.12933	3560	chr14	50499999	52099999	−0.12137	1600
										chr14	52379999	53419999	−0.10299	1040
chr14	76859999	78259999	−0.10653	1400						chr14	76939999	125175837	−0.15227	48,236
chr14	88619999	95699999	−0.17732	7080	chr14	80339999	98619999	−0.31002	18,280					
					chr14	98659999	106899999	−0.14224	8240					
					chr14	106939999	118059999	−0.26608	11,120					
					chr14	118099999	125175837	−0.13545	7076					
					chr15	9419999	10299999	−0.13815	880					
chr15	13459999	23819999	−0.1377	10,360	chr15	13339999	24459999	−0.20317	11,120					
									6360	chr15	13499999	14979999	−0.10807	1480
chr15	46139999	47259999	−0.15127	1120	chr15	44659999	51019999	−0.17027		chr15	19699999	22619999	−0.10222	2920
chr15	47419999	51059999	−0.12808	3640										
					chr15	89459999	90219999	−0.12766	760					
					chr16	59059999	89699999	−0.13622	30,640					
chr17	17499999	22579999	−0.11022	5080	chr17	18299999	20899999	−0.2772	2600					
					chr17	37259999	42659999	−0.17895	5400					
					chr17	50899999	56019999	−0.11015	5120					
					chr17	57539999	62859999	−0.14589	5320					
chr17	76139999	78259999	−0.13494	2120	chr17	76139999	78299999	−0.17557	2160					
					chr17	81499999	83219999	−0.10341	1720					
chr17	89499999	95255954	−0.10669	5756	chr17	89259999	95255954	−0.15303	5996					
chr18	16939999	19899999	−0.11972	2960	chr18	16979999	19779999	−0.1813	2800	chr18	7899999	8339999	−0.13658	440
chr18	26139999	31459999	−0.11968	5320	chr18	26219999	31379999	−0.21126	5160					
chr18	51059999	52299999	−0.14649	1240	chr18	50739999	52659999	−0.19077	1920	chr18	51659999	52099999	−0.12795	440
					chr18	70659999	73179999	−0.1304	2520					
chr18	75979999	76259999	−0.16726	280						chr18	75779999	76499999	−0.11312	720
chr18	85659999	90459999	−0.1178	4800	chr18	85299999	90765552	−0.13801	5466					
					chr19	47779999	52739999	−0.12561	4960					

segments with segment means greater than 0.1 threshold and a size greater than 250 kb. Deletions detected in undifferentiated, TGF-ß differentiation induced and FCS differentiation induced cells were summarized in Table 1.

We used this low threshold of 0.1 for amplification and deletion detection because we were using a mixture of cells. Fluorescence in situ hybridization experiments at a single cell level had shown that gene amplifications were present at various percentages of the cells and in various copy numbers per single cell [2]. For further confirmation of the usefulness of a low threshold we did TaqMan copy number assay for two amplified genes namely *GFAP* and *FZR1*. *GFAP* revealed a 0.347 $\log_2$ ratio and *FZR1* a 0.2 $\log_2$ ratio.

2.3. qPCR analysis

TaqMan Copy Number Assays for genes *GFAP* and *FZR1* were performed following manufacturer's instructions. We used the *TERT* TaqMan Copy Number reference assay for relative quantitation of copy number of target genes. Mouse genomic DNA (Clontech) was used as control standard for normal diploid copy number. TaqMan assays were run in two independent experiments, each in four technical replicates and results were analyzed using StepOne™ Software v2.0 and copy numbers were analyzed using CopyCaller™ software. Mean results of four technical replicates were summarized in Fig. 1a (*GFAP*) and b (*FZR1*). The copy number calculated by Software Copy Caller™ revealed an increased copy number 3-fold of *GFAP* for SFME cell differentiation induced by TGFß for 8 h, 12 h and 24 h. In SFME cell differentiation induced by FCS for 8 h, 12 h and 24 h, the copy number was 2.5, 3 and 2.5-fold respectively. The software also identified an increased copy number of 2.5-fold for *FZR1* for SFME cell differentiation induced by TGFß for 8 h, 12 h and 24 h. Likewise we found an increased copy number of 2.5-fold for SFME cell differentiation induced by FCS for 24 h. These results confirmed our previous array-CGH analysis and FISH experiments. Interestingly the higher $\log_2$ ratio values for *GFAP* in array-CGH experiments corresponded to an elevated copy number value in TaqMan qPCR experiments.

3. Discussion

Here we report detailed information on threshold choice for detection of gene amplification using NimbleGen 730K mouse whole genome array and correlation between $\log_2$ ratio values and copy number values

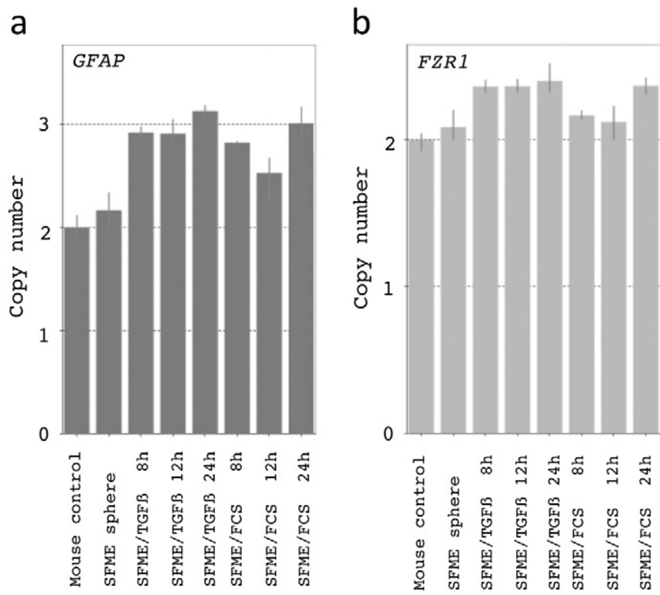

Fig. 1. Amplification analysis using q-PCR. Amplification of *GFAP* and *FZR1* was analyzed by qPCR using the TaqMan copy number assays. SFME cells grown as spheres served as undifferentiated controls. SFME cells were investigated at three different time points with TGF-ß and FCS differentiation induction. Mouse genomic DNA served as standard for normal diploid copy number. The average copy number was 3 of *GFAP* in SFME cell differentiation induced by TGF-ß for 8 h, 12 h and 24 h. In SFME cell differentiation induced by FCS for 8 h, 12 h and 24 h, the average copy number was 2.5, 3 and 2.5 respectively. The average copy number was 2.5 for *FZR1* in SFME cell differentiation induced by TGF-ß for 8 h, 12 h and 24 h, induced by FCS for 24 h. There was no copy number gain for *FZR1* detectable in SFME cell differentiation induced by FCS for 8 h and 12 h.

from TaqMan qPCR experiments. Here and in our previous report we detected a complex pattern of amplifications and deletions. Both amplifications and deletions were only detectable after a low threshold setting. Threshold settings of 0.8 used in many studies were very likely to miss alterations that were present in a subpopulation of the investigated cells. Our confirmation using qPCR strongly argues for a low threshold setting. This dataset is an additional step towards uncovering copy number changes upon differentiation in mammalian stem cells.

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