Data in Brief

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

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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-β 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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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

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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       | log₂ | Size  | Start       | End       | log₂ | Size  |
| chr1   | 110459999  | 112459999 | 0.15437 | 2000  | chr1       | 157699999 | 164399999 | −0.11251 | 6640  |
|        | 125099999  | 125779999 | 0.1133  | 680   | chr1       | 179699999 | 180099999 | −0.17032 | 400   |
| chr1   | 157499999  | 166019999 | −0.11672 | 8520  | chr1       | 154599999 | 157799999 | −0.2969  | 320   |
| chr2   | 85619999   | 88979999  | −0.1151  | 4360  | chr2       | 80059999  | 81099999  | −0.14237 | 1040  |
| chr2   | 94819999   | 101179999 | −0.10144 | 6360  | chr2       | 93099999  | 94059999  | −0.18368 | 360   |
| chr2   | 140259999  | 140739999 | 0.00916  | 480   | chr2       | 110219999 | 115099999 | −0.16231 | 4880  |
| chr2   | 174619999  | 176799999 | −0.08189 | 2360  | chr2       | 116859999 | 120779999 | −0.1109  | 3920  |
| chr3   | 107799999  | 152199999 | −0.10217 | 4440  | chr3       | 123399999 | 127819999 | −0.16916 | 4760  |
| chr3   | 152599999  | 158199999 | −0.25966 | 562   | chr3       | 123059999 | 127819999 | −0.16916 | 4760  |
| chr3   | 232199999  | 236299999 | −0.26682 | 2280  | chr3       | 125819999 | 127739999 | −0.16144 | 1920  |
| chr3   | 47419999   | 48019999  | −0.20803 | 600   | chr3       | 125819999 | 128999999 | −0.24645 | 5100  |
| chr3   | 71339999   | 73539999  | −0.1502  | 2200  | chr3       | 123339999 | 125779999 | −0.35956 | 2440  |
| chr3   | 122399999  | 125779999 | −0.26682 | 2280  | chr3       | 123399999 | 125779999 | −0.35956 | 2440  |
| chr3   | 125819999  | 128999999 | −0.16294 | 3120  | chr3       | 125819999 | 128999999 | −0.16294 | 3120  |
| chr4   | 75659999   | 80779999  | −0.2069  | 5120  | chr4       | 80059999  | 81099999  | −0.14237 | 1040  |
| chr4   | 80779999   | 80779999  | −0.2069  | 5120  | chr4       | 80059999  | 81099999  | −0.14237 | 1040  |
| chr4   | 89259999   | 94339999  | −0.13013 | 5080  | chr5       | 93099999  | 94059999  | −0.18368 | 360   |
| chr4   | 94339999   | 94339999  | −0.13013 | 5080  | chr5       | 93699999  | 94059999  | −0.18368 | 360   |
| chr4   | 138299999  | 140059999 | −0.14334 | 1760  | chr5       | 118599999 | 195399999 | −0.12821 | 7680  |
| chr4   | 140059999  | 140059999 | −0.14334 | 1760  | chr6       | 54859999  | 61859999  | −0.20136 | 7000  |
| chr4   | 138299999  | 140059999 | −0.14334 | 1760  | chr6       | 41539999  | 47339999  | −0.10686 | 5840  |
| chr7   | 106999999  | 121199999 | −0.24888 | 1440  | chr7       | 78299999  | 91059999  | −0.10839 | 12,760|
| chr7   | 78299999   | 91059999  | −0.10839 | 12,760| chr7       | 81459999  | 81739999  | −0.14919 | 280   |
| chr8   | 99539999   | 106019999 | −0.10809 | 6480  | chr8       | 71699999  | 72019999  | −0.1087  | 320   |
| chr9   | 35659999   | 35939999  | −0.21324 | 280   | chr9       | 35659999  | 36059999  | −0.16676 | 400   |
| chr9   | 37899999   | 38899999  | −0.10023 | 1200  | chr9       | 37419999  | 39759999  | −0.10149 | 2560  |
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 SMFE cell differentiation induced by TGFβ for 8 h, 12 h and 24 h. In SMFE 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 SMFE cell differentiation induced by TGFβ for 8 h, 12 h and 24 h. Likewise we found an increased copy number of 2.5-fold for SMFE cell differentiation induced by FCS for 24 h. These results confirmed our previous array-CGH analysis and FISH experiments. Interestingly the higher log2 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 log2 ratio values and copy number values

| Sphere | Start | End | log2 | Size |
|--------|-------|-----|------|------|
| ch10   | 35579999 | 35939999 | −0.17695 | 360 |
| ch10   | 45899999 | 51019999 | −0.12617 | 5120 |
| ch10   | 100819999 | 105099999 | −0.16547 | 428 |
| ch10   | 128539999 | 129975647 | −0.10464 | 1436 |
| ch11   | 36019999 | 42459999 | −0.11313 | 6440 |
| ch14   | 76859999 | 78259999 | −0.10653 | 1400 |
| ch14   | 88619999 | 95699999 | −0.17732 | 7080 |
| ch15   | 13459999 | 23819999 | −0.13777 | 10360 |
| ch15   | 46139999 | 47259999 | −0.15127 | 1120 |
| ch15   | 47419999 | 51059999 | −0.12808 | 3640 |
| ch17   | 17499999 | 22579999 | −0.11022 | 5080 |
| ch17   | 76139999 | 78259999 | −0.13494 | 2120 |
| ch17   | 89499999 | 95259594 | −0.10669 | 5756 |
| ch18   | 16939999 | 19889999 | −0.11972 | 2960 |
| ch18   | 26139999 | 31459999 | −0.11968 | 5320 |
| ch18   | 51059999 | 52299999 | −0.14649 | 1240 |
| ch18   | 75979999 | 76259999 | −0.16726 | 280 |
| ch18   | 85659999 | 90459999 | −0.11787 | 4800 |
| ch19   | 47779999 | 52739999 | −0.12561 | 4960 |

Table 1 (continued)
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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References

[1] U. Fischer, A. Keller, C. Backes, E. Meese, Genome-wide copy number profiling to detect gene amplifications in neural progenitor cells. Genomics Data 2 (2014) 162–165. http://dx.doi.org/10.1016/j.gdata.2014.06.020.

[2] U. Fischer, et al., Gene amplification during differentiation of mammalian neural stem cells in vitro and in vivo. Oncotarget 6 (9) (2015) 7023–7035.