**Introduction**

DNA sequence amplification describes any event that increases the copy number of a gene per haploid genome above the number that is characteristic for that organism [1]. Gene amplification has been reported for defined stages during normal development of Xenopus laevis, Drosophila melanogaster, Sciara coprophila, and Tetrahymena thermophila [1-4]. The ability of a cell to amplify genes represents an alternative to the problem of how best to meet a short and sharp, but heavy demand for a stage-specific protein [1]. This amplification process is spatially and temporally restricted on specific DNA regions and narrow windows of developmental time [4]. In humans, gene amplification has only been found in multidrug resistant cells and in cancer cells. The only circumstantial evidence for gene amplification in normal mammalian cells stems from two studies on mouse embryo cells with double minute chromosomes (DMs), that are cytogenetic manifestations of gene amplification. One study described DMs in cell lines derived from mouse fetus [5]. The other study described DMs in 1% of serum free mouse embryo (SFME) cells and an elevated frequency of DMs in cells grown in medium containing fetal calf serum (FCS) [6]. SFME cells were distributed from the American Type Culture Collection (ATCC) as neural stem cell line. From today’s view, these SFME cells were neural progenitor cells that are capable of differentiating into astrocytes when grown in the presence of growth factor TGF-ß or fetal calf serum (FCS). This hint for gene amplification as physiological process in mammalian cells, specifically progenitor cells, prompted us to study gene amplification in normal human neural progenitor cells (NHNP). These cells grow as spheres and express β-III Tubulin (neuronal lineage) and GFAP (astrocyte lineage) upon differentiation. Recent studies on various human embryonic stem cells revealed genetic changes during prolonged culture [7]. Whole-genome genotyping analysis of NHNP cells created from NHNP primary cells at passage 20 were, however, still considered “normal” with a low number of CNVs (copy number variations)>200 kb (0-2 per line) [8,9]. Notably, NHNP cells used in this study were primary cells in passage 1 [8].

The results of our genome-wide gene amplification analysis during *in vitro* differentiation of normal human progenitor cells revealed a complex amplification pattern after two and five days of differentiation. Representative examples of amplified chromosomal regions were confirmed by fluorescence in situ hybridizations. We further characterized those cells with amplifications using immunofluorescence staining. We found a strong overlap of amplified genes in neural progenitor cells undergoing differentiation and amplified genes in malignant tumors derived from astrocytes.

**Results**

Identification of gene amplifications using array-CGH analysis

Differentiation of NHNP cells was induced by withdrawal of EGF and bFGF and supplementation of brain derived neurotrophic factor (BDNF). The expression of Tubulin beta-3 chain and GFAP was analyzed by immunofluorescence after 24 h following differentiation induction. For amplification analysis total DNA was isolated from undifferentiated NHNP sphere cells and from NHNP cells differentiated for 24 h, 2 d and 5 d
Table 1. Overview on amplified chromosomal regions.

| Genes | Chromosome start | end | log2 ratio | size (Mb) |
|-------|------------------|-----|------------|-----------|
| chr1  | 362499           | 3837499 | 0,194   | 3,48     |
| chr1  | 5612499          | 12612499 | 0,115   | 7,00     |
| chr1  | 151587499        | 155337499 | 0,125   | 3,75     |
| chr1  | 14362499         | 46587499 | 0,103   | 32,23    |
| chr1  | 200612499        | 204962499 | 0,136   | 4,35     |
| chr2  | 19937499         | 20737499 | 0,126   | 0,80     |
| chr2  | 220037499        | 220312499 | 0,153   | 0,28     |
| chr3  | 46637499         | 50112499 | 0,117   | 3,48     |
| chr3  | 50137499         | 50687499 | 0,239   | 0,55     |
| chr3  | 51912499         | 52862499 | 0,164   | 0,95     |
| chr3  | 185087499        | 186037499 | 0,122   | 0,95     |
| chr3  | 194887499        | 196812499 | 0,104   | 1,93     |
| chr3  | 37499            | 9362499  | 0,103   | 9,33     |
| chr3  | 40362499         | 44862499 | 0,100   | 4,50     |
| chr4  | 137499           | 2987499  | 0,140   | 2,85     |
| chr7  | 43962499         | 45087499 | 0,127   | 1,13     |
| chr7  | 72012499         | 75887499 | 0,113   | 3,88     |
| chr7  | 141837499        | 142212499 | 0,186   | 0,38     |
| chr7  | 150237499        | 150737499 | 0,166   | 0,50     |
| chr8  | 27162499         | 27587499 | 0,127   | 0,43     |
| chr8  | 142112499        | 142612499 | 0,124   | 0,50     |
| chr8  | 143087499        | 146257115 | 0,120   | 3,17     |
| chr9  | 115812499        | 116412499 | 0,130   | 0,60     |
| chr9  | 122237499        | 140235768 | 0,104   | 18,00    |
| chr10  | 72962499         | 73787499 | 0,157   | 0,83     |
| chr10  | 79237499         | 81362499 | 0,125   | 2,13     |
| chr11  | 62499            | 4162499  | 0,112   | 4,10     |
| chr11  | 60737499         | 61387499 | 0,206   | 0,65     |
### Table 1. Cont.

| Chromosome start | end     | log2 ratio | size (Mb) | Chromosome start | end     | log2 ratio | size (Mb) | Genes       |
|------------------|---------|------------|-----------|------------------|---------|------------|-----------|-------------|
| chr11            | 61912499| 62412499   | 0.205     | 0.50            |
| chr11            | 63362499| 63812499   | 0.258     | 0.45            |
| chr11            | 63837499| 64112499   | 0.140     | 0.28            |
| chr11            | 64412499| 64887499   | 0.145     | 0.48            |
| chr11            | 65312499| 66587499   | 0.174     | 1.28            |
| chr12            | 6237499 | 7212499    | 0.136     | 0.98            |
| chr12            | 123362499| 123687499 | 0.213     | 0.33            |
| chr12            | 123712499| 124462499 | 0.110     | 0.75            |
| chr12            | 12499 | 24037499   | 0.117     | 1.98            |
| chr12            | 76412499| 77062499   | 0.145     | 0.65            |
| chr15            | 18537499| 20062499   | 0.139     | 1.53            |
| chr15            | 29187499| 29462499   | 0.128     | 0.28            |
| chr15            | 30237499| 30612499   | 0.126     | 0.38            |
| chr15            | 7112499 | 73037499   | 0.160     | 0.93            |
| chr15            | 75687499| 76137499   | 0.202     | 0.45            |
| chr15            | 80387499| 80862499   | 0.186     | 0.48            |
| chr15            | 12499 | 1487499    | 0.221     | 1.48            |
| chr16            | 29487499| 31512499   | 0.151     | 2.03            |
| chr16            | 82487499| 83737499   | 0.106     | 1.25            |
| chr16            | 83762499| 8437499    | 0.222     | 0.58            |
| chr16            | 86587499| 88707518   | 0.181     | 2.12            |
| chr16            | 12499 | 1487499    | 0.221     | 1.48            |
| chr16            | 76412499| 77062499   | 0.145     | 0.65            |
| chr17            | 16037499| 19787499   | 0.138     | 3.75            |
| chr17            | 23787499| 24537499   | 0.182     | 0.75            |
| chr17            | 33687499| 36012499   | 0.119     | 2.33            |
| chr17            | 55437499| 56687499   | 0.148     | 1.25            |
| chr17            | 64662499| 69387499   | 0.121     | 4.73            |
| chr17            | 73762499| 74137499   | 0.151     | 0.38            |
| chr17            | 79962499| 80287499   | 0.161     | 0.33            |
| chr17            | 82487499| 83712499   | 0.127     | 1.23            |
| chr17            | 83737499| 84412499   | 0.250     | 0.68            |
| chr17            | 85862499| 88707518   | 0.162     | 2.85            |
| chr17            | 12499 | 2937499    | 0.124     | 2.93            |
| chr17            | 6682499 | 8337499    | 0.139     | 1.48            |
| chr17            | 16262499| 18037499   | 0.144     | 1.78            |
| chr17            | 22587499| 24437499   | 0.101     | 1.85            |
| chr17            | 33687499| 36012499   | 0.119     | 2.33            |
| chr17            | 36812499| 40937499   | 0.122     | 4.13            |
| chr17            | 41037499| 41537499   | 0.104     | 0.50            |
| chr17            | 46087499| 46687499   | 0.113     | 0.60            |
| chr17            | 67587499| 76512499   | 0.126     | 8.93            |
| chr17            | 76537499| 76937499   | 0.291     | 0.40            |
| chr18            | 76962499| 78637061   | 0.149     | 1.67            |
| chr18            | 54687499| 54962499   | 0.126     | 0.28            |
| chr18            | 33037499| 33462499   | 0.103     | 0.43            |
| chr18            | 75287499| 76107311   | 0.104     | 0.82            |
| chr19            | 212499 | 8487499    | 0.145     | 8.28            |
| chr19            | 9737499 | 19837499   | 0.131     | 10.10           |
| chr19            | 49912499| 50537499   | 0.199     | 0.63            |
| chr19            | 37762499| 47887499   | 0.101     | 10.13           |
respectively and analyzed on NimbleGen 720K human whole genome tiling arrays. 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 q spline normalization. Following normalization a 10 x window–averaging step is applied. Window-averaging reduces the size of the data and reduces the noise in the data. For amplification 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. Chromosomal regions that revealed copy number gains and match CNVs (copy number variations) present in the Database of Genomic Variants available at UCSC Genome Browser were excluded from further analysis. While we did not detect amplified regions in NHNP cells at zero time and 24 h after differentiation, we found numerous chromosomal regions with copy number gains in NHNP cells after 2 d and 5 d of differentiation. In total we found 66 amplified chromosome regions after 2 d of differentiation and 95 amplified chromosome regions after 5 d of differentiation (Table 1). We also detected 9 deleted chromosome regions after 2 d of differentiation and 30 deleted chromosome regions after 5 d of differentiation. Whole genome profiles were presented in Figure 1.

**Confirmation of gene amplification of selected loci**

Using array-CGH we identified amplified chromosome regions in a mixed population of cells during *in vitro* differentiation. To validate these results we used fluorescence in situ hybridization (FISH) on loci with log₂ ratios ranging from 0.126 to 0.250. We analyzed one gene for each locus including 1q23.1 at 154.98 Mb (HDGF); 1q32.1 at 202.35 Mb (SOX13), 12q14.1 at 56.43 Mb (CDK4), at 56.44 Mb (CIP27B1) and at 56.63 Mb (ARCC6BP1/KUB3), 12q24.31 at 121.26 Mb (DIABLO), 16q24.1 at 84.27 Mb (GINS2), 17p13.1 at 7.52 Mb (TP53) and 17q21.31 at 40.4 Mb (C1QL1). FISH analysis confirmed the amplifications for all loci (Figure 2). Next we determined the amplification frequency analyzing 150 nuclei per locus. We found an average amplification frequency for GINS2 and CDK4 of 5% after 2 d of differentiation, an amplification frequency for CIP27B1 of 3% after 2 d of differentiation, an amplification frequency for SOX13, C1QL1 and HDGF of 10% after 5 d of differentiation and an amplification frequency for TP53 and DIABLO of 5% after 7 d of differentiation. Both the copy number variation in cells with gene amplification, and the absence of gene amplifications in many cells, account for the low increase in log₂ ratio in array-CGH analysis.

As further validation step we compared the amplification event between genes from two neighboring chromosome regions. Within chromosome region 16q24.1 the log₂ ratio values revealed an increase of genomic sequences at 83.7–84.4 Mb. In the same chromosome region, the log₂ ratio values indicated a normal copy number at 82 Mb (Figure 3A). FISH experiments revealed amplification of the GINS2 gene that maps at 84.27 Mb, but no amplification for CDH13 at 82 Mb (Figure 3B). FISH analysis also provided evidence for a large heterogeneity of amplifications in neighboring cells. Figure 3Bi shows a nucleus with amplified GINS2 fluorescence signals next to a nucleus with only 5 GINS2 specific fluorescence signals.

For further validation we analyzed chromosome region 12q14.1. For this region the log₂ ratio values indicated an increase of genomic sequences at 54–56.7 Mb (Figure 3C). FISH confirmed not only the amplification of the CDK4 gene at 56.43 Mb and the adjacent ARCC6BP1/KUB3 gene at 56.63 Mb (Figure 3D) but also indicated the difference in the localization of the amplified sequences between both genes. While the CDK4 specific fluorescence signals were widely spread, the ARCC6BP1/KUB3 signals were more focused. (Figure 3 D ii).

**Characterization of cells with gene amplifications**

We asked whether the identified amplification pattern was different between cells that were still part of the sphere and cells that migrated out of the sphere during the differentiation process. By using simultaneous FISH and immunofluorescence (IF) we analyzed the amplification status of selected genes and the expression of the differentiation marker GFAP. After two or five
days of differentiation we found both NHNP cells with weak GFAP staining close to the nucleus and NHNP cells with a strong GFAP staining throughout the cytoplasm of the cell body and the appendages. This staining pattern was reproducible in several biological replicates and indicates different stages of the differentiation process at the two time points.

While cells with strong GFAP expression showed a normal copy number for all genes tested, e.g. CDK4 and GINS2, cells with weak GFAP expression showed amplifications of CDK4 and GINS2 after 2 d and 5 d (Figures 4 A, B). While cells with a weak GFAP expression were localized in or near the sphere, cells that had migrated out of the sphere revealed a stronger expression of the differentiation marker GFAP and a differentiated morphology of the cell body with typical appendages. Similar results were obtained with immunofluorescence staining using the differentiation marker Tubulin beta-3 chain. Notably, after 11 days of differentiation we still found CDK4 amplification in NHNP cells with weak Tubulin beta-3 chain expression (Figure 4 C, D).

**Discussion**

SFME cells are non-tumorigenic and display characteristics of progenitor cells of the central nervous system [10]. Addition of FCS up-regulates GFAP expression indicating the capacity to differentiate into astrocytes [10]. The SFME cell line had undergone 96 population doublings in serum free medium and revealed double minutes in one percent of the cells [6]. A higher percentage of double minutes were detectable in SFME cells that were capable of growing in FCS containing medium. This increase in percentage of cells with double minutes under differentiation promoting conditions prompted us to investigate gene amplification during differentiation.

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**Figure 1. Whole chromosome plots.** A genome-wide view of the $10^6$ window-averaged data at 25 kb resolution is displayed for NHNP cells at day 0, day 1, day 2 and day 5 during differentiation. doi:10.1371/journal.pone.0037422.g001
Here we decided to use human neural progenitor cells that were grown as spheres and that were capable of differentiating into neurons and astrocytes. There is early evidence that cells with glial morphology migrate out of the spheres as result of an induced differentiation [11]. A more recent study reported human NPCs (neural progenitor cells) that expressed both GFAP and Tubulin beta-3 chain in the migration area 24 h after differentiation induction [12]. NHNP sphere cells used in this study revealed cells with glial morphology that migrated out of the sphere a short time after differentiation induction. We were able to confirm GFAP and Tubulin beta-3 chain expression in the migration area 24 h after differentiation induction. In our study gene amplifications appear to occur preferentially in cells that still localize in or close to the sphere. From gene amplification analysis in glioblastoma cells it is known that amplifications can be lost [13]. In this study we can raise the hypothesis that cells with amplifications die upon differentiation or that differentiated cells have lost their amplifications. Further investigations will be necessary to determine whether neural progenitor cells show amplification as a prerequisite for differentiation or whether the differentiation process is the prerequisite for amplification.

Since small focal gains likely represent copy number variations that are commonly found in the human genome, we considered only loci larger than 250 kb. Our amplification analysis after two and five days of in vitro differentiation revealed a complex genome-wide amplification pattern with 66 or 93 amplified loci. The size of the amplified chromosome regions was between 250 kb and more than 10 Mb. Array-CGH data were carefully interpreted. We are aware that a gain in log2 ratio value of 0.25 can be regarded as gain of one copy. But these calculations were only true when analyzing a homogenous cell population. Here we analyzed a very complex cell population with many cells in differing stages of differentiation. Even after 7 d or 11 d of differentiation we still find cells with amplifications and cells with a weak GFAP or Tubulin ß III staining likely indicating early stages of the differentiation process in these cells. It is very likely that we still missed amplifications in other chromosomal regions by our array-CGH approach because the number of cells with those amplifications is too small or the level of amplification is not high enough for detection by this means. This is further supported by the fact, that in our array-CGH analysis CDK4 gene amplifications were only detected after 5 d of differentiation. But FISH analysis on NHNP cells differentiated for 2 d already revealed CDK4 amplifications as shown in Figure 2, 3.

Analysis of these chromosome regions revealed hundreds of genes that were involved in this amplification process. Besides amplifications, we also detected deletions that were mainly localized in chromosome regions lacking genes or contain only few genes. In contrast, amplified chromosome regions mainly map within chromosome regions with a high gene density (Figure 5).

As stated above, gene amplification is a hallmark of many human tumors including brain tumors. Several chromosomal regions amplified in neural progenitor cells contain genes that are also amplified in glioblastoma including CDK4, SOX13, TERT, ABCC3, RANBP1, MDH2, CAMK2A, ID2 and FUT1 [14–18]. In

Figure 2. FISH analysis of amplified loci. For each FISH analysis, a BAC or cosmid clone containing the indicated gene was Cy3-labelled (pink) and hybridized against fixed NHNP cells that were differentiated for either 2, 5 or 7 days. Amplifications are shown for C1QL1 RP11-113A24 (5 days), SOX13 RP11-66D17 (5 days), HDGF RP11-118F19 (2 days), GINS2 RP11-1081A10 (7 days), DIABLO RP11-568C23 (7 days). Nuclei were counterstained with DAPI. Size calibration bar = 5 μm. Notably, the degree of amplification various within each analysis due to the high heterogeneity of the amplifications in each cell population.

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addition, we found an overlap between chromosomal regions amplified in NHNP cells that were differentiated for 2 and 5 days, and gained chromosome regions in 251 glioblastoma deposited in the TCGA data collection [19].

Gene amplifications in normal human cells as physiological process have not been reported yet. Since our in vitro study was focused on a narrow time window of few days during the differentiation process and since amplifications were found only in a smaller number of cells, we would not expect to see readily identifiable amplification events in human normal tissue. Further light onto the amplification process will be shed by comparative analyses of this process in other mammals to see if and to what extent amplification can be found during cell differentiation in other species.

Figure 3. Detailed gene amplification analysis on human chromosome 16 and 12. Representative sections of log2 ratio profiles for undifferentiated (0 d) NHNP cells and cells that were differentiated for 2 and 5 days. Base count is given on the x-axis and log2 ratio on the y-axis for chromosome 16q24.1 (A) and 12q14.1 (C). Chromosomal localization of BAC probes used for FISH were indicated at the bottom of figures C and D. A GINS2 specific BAC probe that was labeled in pink and a CDH13 specific BAC probe that was labeled in green were hybridized simultaneously against fixed NHNP cells that were differentiated for 2 days. GINS2 amplification is indicated as pink speckled fluorescence signals whereas the neighboring CDH13 gene shows only single copy fluorescence signals (Bi). Neighboring cells with and without GINS2 amplification are shown in Figure Bii. A CDK4 specific BAC probe that was labeled in pink and a XRCC6BP1/KUB3 specific BAC that was labeled in green were hybridized simultaneously against NHNP cells that were differentiated for 2 days. CDK4 and KUB3 amplifications were detectable as cluster of pink and green speckled fluorescence signals. CDK4 specific signals spread over a more extended area than the KUB3 specific signals (D). Nuclei were counterstained with DAPI (B). Size calibration bar = 5 μm.

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Materials and Methods

Cell culture and differentiation

NHNP cells were obtained from Lonza (Verviers, Belgium) as cryopreserved sphere culture and were cultivated in maintenance medium (NPMM) containing EGF and bFGF for 24 h after thawing. NHNP cells were primary cells in P1 established from human embryonic brain (17WG, male and 18WG, sex unknown). Spheres were not expanded or cultured after these initial 24 h. Spheres were seeded on laminin-coated glass slides ( autoclaved) and allowed to attach to the surface for 5 minutes. Spheres were differentiated using differentiation medium (NPDM) supplemented with BDNF (25 ng/ml).
DNA preparation

Genomic DNA was extracted from cell cultures and normal blood lymphocytes using NaCl/chloroform extraction. Control genomic DNA was mixed from male and female healthy blood lymphocytes to minimize for normal CNV detection.

Array preparation, hybridization and detection

NimbleGen 3x720K whole genome array hybridization was done using the certified full service of NimbleGen 3x720K human whole genome array hybridization from ImaGenes Berlin, Germany. Detailed information on data analysis is described at http://www.nimblegen.com/products/cgh/wgt/human/3x720k/index.html. Array-CGH analysis was performed on primary NHNP cells at time point zero, and after 1 d, 2 d and 5 d of differentiation. The array-CGH experiments were done with independently derived primary cells. Array data were deposited in GEO (GSE30636).

Fluorescence in Situ Hybridization and Immunofluorescence staining

BAC clones were from RP-11 (http://www.chori.org/bacpac/) libraries of the Welcome Trust Sanger Institute and cosmid clone for C1P27B1 (LLNLc132M0263Q2) available from ImaGenes GmbH, Germany.

BAC probes were directly labeled using High Prime Labeling System (Roche Molecular Biochemicals, Germany). 1 µg of BAC-DNA was labeled with Cyanine-3-dCTP (Cy3) or Cyanine-5-dCTP (Cy5) (PerkinElmer, Germany), according to the manufacturers instructions. 60 ng of Cy3-labeled and/or Cy5-labeled probe DNA were precipitated in the presence of human Cot-1 DNA. Samples were resuspended in hybridization mix (50% formamide, 2×SSPE, 10% dextran sulphate and 4% SDS).

Hybridization

Labeled BAC probes were applied to the slides and denatured for 2 min. at 80°C. Hybridization was done in a humid chamber at 37°C for 16 h. Post hybridization washes were performed in 50% formamide/2×SSPE (4×5 minutes; 45°C) followed by 0.1× SSPE (3×5 minutes) at 60°C. Nuclei were counterstained with DAPI (4’6’-Diamidino-2-phenylindole) (1 µg/ml in PBS) for 4 minutes and mounted with VectaShield mounting medium.

NHNP sphere cells were grown on laminin-coated slides under differentiation promoting conditions. Slides were washed once with PBS and methanol fixed 10 min at −20°C. Slides were treated for 5 min in 0.02% Tween-20/PBS.

For FISH with simultaneous immunofluorescence staining, slides were RNase treated (100 µg/ml RNaseA in 2×SSC) for 15 minutes at 37°C. Postfixation was done by 1% formaldehyde/1×PBS for 10 minutes at room temperature. Slides were blocked with goat serum and incubated for 1 h with antibodies either chicken polyclonal to GFAP (ab4674, Abcam) or rabbit polyclonal to neuron specific beta-III-Tubulin (ab18207, Abcam) and detected using Alexa-488 coupled secondary antibodies. Finally, slides were dehydrated by an ascending ethanol series (70%/80%/96%) and air-dried.

For FISH without simultaneous immunofluorescence staining, slides were RNase treated (100 µg/ml RNaseA in 2×SSC) for 30 minutes at 37°C and pepsin treated (0.005% in 0.01 M HCl at 37°C) for 10 minutes. Postfixation and dehydration was done as described.

Both immune fluorescence analyses with and without FISH were done in biological replicates with primary cell cultures that were independently derived from human embryonic brain.

Figure 4. FISH and immunofluorescence analysis. FISH with GINS2 specific BAC (pink) and simultaneous immunofluorescence staining with GFAP (green) revealed GINS2 amplification in cells with beginning GFAP expression after 5 d of differentiation (A). FISH with CDK4 specific BAC (pink) and immunofluorescence staining with GFAP revealed CDK4 amplification in cells with beginning GFAP expression after 2 d of differentiation (B). FISH with CDK4 specific BAC (pink) and immunofluorescence staining with Tubulin-ß-III-chain (green) revealed CDK4 amplification in cells with beginning Tubulin-ß-III-chain expression after 11 d of differentiation (C and D). Nuclei were counterstained with DAPI. Size calibration bar = 5 µm.

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Figure 5. Correlation of gene content to chromosome region. The correlation is shown for human chromosome 16 with several amplified and one deleted region in NHNP cells after 5 d differentiation. The log2 ratio profile of the 10× window averaged data is presented at 25 kb resolution (A). Fused lasso analysis is performed using the same data points (B). GC repeats, gene content and banding pattern is shown as indicated by Ensembl genome browser (C). Both the log2 ratio profile and the fused lasso analysis indicate an overlap between amplifications and regions with high GC and gene content and an overlap between deletion and regions with low GC and gene content.
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Author Contributions
Conceived and designed the experiments: UF CW EM. Performed the experiments: UF MV. Analyzed the data: AK CB. Wrote the paper: UF EM.

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