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Comprehensive characterization of genomic instability in pluripotent stem cells and their derived neuroprogenitor cell lines

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ABSTRACT

The genomic integrity of two human pluripotent stem cells and their derived neuroprogenitor cell lines was studied, applying a combination of high-resolution genetic methodologies. The usefulness of combining array-comparative genomic hybridization (aCGH) and multiplex fluorescence in situ hybridization (M-FISH) techniques should be delineated to exclude/detect a maximum of possible genomic structural aberrations. Interestingly, in parts different genomic imbalances at chromosomal and subchromosomal levels were detected in pluripotent stem cells and their derivatives. Some of the copy number variations were inherited from the original cell line, whereas other modifications were presumably acquired during the differentiation and manipulation procedures. These results underline the necessity to study both pluripotent stem cells and their differentiated progeny by as many approaches as possible in order to assess their genomic stability before using them in clinical therapies.

1. Introduction

Genomic structural alterations in stem cells have been reported since the first embryonic stem cell lines were developed in primates by Thomson and Marshall (1998) and Thomson et al. (1998). Many other such examples were subsequently reported, including structural abnormalities like 46,X,idic(X)(q21) (Inzunza et al., 2004) and 47,XX, del(7)(q11.2),+i(12)(p10) (Imreh et al., 2006), or numerical ones like aneuploidies found in up to 20% of the studied cells (Rosler et al., 2004). Furthermore, CTG repeat instability, variations in copy number, gain and loss of heterozygosity, epigenetic changes and karyotypic instability induced by cell culture conditions in human stem cell lines have been repeatedly described (De Temmerman et al., 2008; Thomson et al., 2008; Catalina et al., 2008; Dahl et al., 2008; Liang et al., 2008; Närvä et al., 2010).

Of special interest among stem cells are induced pluripotent stem cells (iPSC), which add the potential risk aris\ing from the methods used to derive them from somatic cells to those intrinsic to the manipulation and expansion of any stem cell in vitro. Thus, chromosomal monosomy induced by modified stem cells used in therapy was recently described (Dunbar and Larochelle, 2010). Similarly, Hussein et al. (2011) reported a higher number of copy number variants (CNVs) in iPSC cells at early passages of cell culture, potentially as a result of selection during reprogramming to pluripotency.

Genomic anomalies can be found in any type of pluripotent cell, regardless of culture conditions, manipulation or modifications. However, there is a significant lack of information about genomic instabilities and variations that could potentially be found in differentiated derivatives of pluripotent cell types. If those cells would be used either in cell therapy or as a model in basic research, it is important that their genomic integrity be analyzed and characterized. Intuitively, the idea that such variants could accumulate in the relatively short time spanning the completion of a typical differentiation protocol (1–3 weeks) could be easily dismissed (Winkler et al., 2009; Stephenson et al., 2010).

Our data, however, indicate that this precisely could be the case. Preliminary evidence was obtained by application of multiplex-fluorescence in situ hybridization (M-FISH; Speicher et al., 1996) and array comparative genomic hybridization (aCGH; Shinawi and Cheung, 2008) on both, pluripotent cell lines (iPSC and human embryonal stem cells = ESC) and their neuroprogenitor cell (NPC) derivatives; i.e. genomic abnormalities and variants may indeed appear as a result of in vitro differentiation.

2. Material and methods

2.1. Pluripotent stem cells (iPSC and ESC) and derived NPC cells

Here two human pluripotent stem cell line (iPSC and ESC) and one corresponding neuroprogenitor cell line (NPC) derivative were studied: the different iPSC and hESC and their corresponding derived NPC were obtained from the National Laboratory of Stem Cell, in Rio de Janeiro, Brazil. These cell lines were developed from a normal

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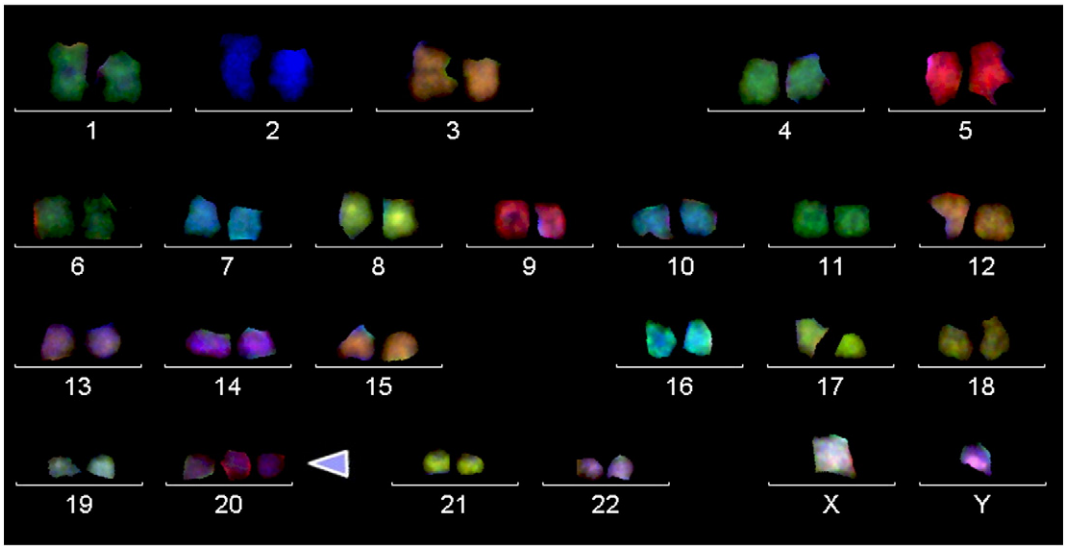

Fig. 1. M-FISH result of cell line NPC derived from ESC showing a karyotype 47,XY,+20. Trisomy 20 is highlighted by an arrowhead.

individual (ESC) and from a schizophrenic patient (iPSC). Those cells lines were established following standard procedures and are described in Fraga et al. (2011) (ESC) and Paulsen et al. (in press) (iPSC).

2.2. Cytogenetics and molecular cytogenetics

Cultured cells were prepared according to cytogenetic standard protocols. Chromosome suspensions were dropped onto slides as described in Claussen et al. (2002). M-FISH was done using home-made whole chromosome painting probes according to Weise et al. (2002). 100–200 metaphases were acquired per case by a fluorescence microscope (Axioplan 2, Zeiss, Germany) with a PCO VC45 CCD camera (PCO, Kehl, Germany) using isis-software (MetaSystems, Altlussheim, Germany) for evaluation. Between 17 and 39 metaphases were evaluable and included into the results.

2.3. Array comparative genomic hybridization

aCGH was performed using an Agilent Platform with a 180k chip according to manufacturer's protocol (Agilent Technologies, Santa Clara, CA, USA). Experiments were conducted using a gender-matched genomic DNA pool as reference. The quality of the experiment was evaluated with the QC metric provided by the CGH analysis software (CGH Analytics 3.5.14, Agilent Technologies). Data were analyzed using two different software packages (Blue Genome-Blue Fuse and Agilent) to determine the statistical validity of the findings.

3. Results

All four cell lines could be analyzed by array-CGH. For M-FISH analysis only ESC (pluripotent cell line from normal male) did not yield any usable metaphase spreads.

3.1. M-FISH results

M-FISH analysis revealed a high degree of chromosomal instability in the preparations of the pluripotent cells and their derivatives. Of the acquired metaphases (100–200/line), only a subset was suitable for detailed analyses due to clumsiness, and a significant incidence of single cell aberrations was found in those.

The original iPSC line showed a normal 46,XX[cp17] karyotype with no structural chromosomal aberrations as detected by M-FISH and inverted DAPI-banding. Non-clonal chromosome losses were detected in 11 of the 17 metaphases (Table 1). As summarized in Table 2, the NPC derived from iPSC had a karyotype of 46,XX[cp39], as well. M-FISH and inverted DAPI-banding revealed no structural chromosomal aberrations here, as in the original cell line.

As mentioned above, no metaphase spreads were available for FISH analysis for the induced pluripotent cell line ESC. However, according to the analysis of its derived cell line iPS3-derived NPC2, a normal karyotype should be present most likely in the original source. In contrast, the ESC-derived NPC had a 47,XY,+20[cp10]/46,XY[cp13] karyotype without structural chromosomal aberrations (Fig. 1). A description of the non-clonal chromosome losses detected in 14 of the 33 analyzed metaphases is presented in Table 3.

Table 1
M-FISH results by chromosome for cell line iPSC.

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
11 metaphases showed loss of chromosomes n times	0	0	0	1	0	0	1	0	1	1	0	0	1	0	0	0	1	2	2	4	1	1	0
Composite karyotype	46,XX[cp17]																						

Table 2
M-FISH results by chromosome for cell line NPC derived from iPSC.

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
21 metaphases showed loss of chromosomes n times	6	3	0	0	2	3	2	4	1	4	3	3	4	2	2	0	3	4	3	4	1	6	3
Composite karyotype	46,XX[cp39]																						

Table 3
M-FISH results by chromosome for cell line NPC derived from ESC.

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
14 metaphases showed loss of chromosomes n times	1	0	1	0	1	3	3	1	3	3	0	5	2	1	2	5	1	2	2	4	0	4	0
Composite karyotype	47,XY,+20[cp10]/46,XY[cp13]																						

Overall, M-FISH revealed the gain of chromosome 20 in a subset of one of the four studied cell lines. This trisomy 20 was present in >40% of the analyzed dividing cells [(47,XY,+20[10]/46,XY[13)].

Interestingly, this cell clone was not detected in the aCGH test (see below).

3.2. aCGH results

Overall fourteen different genomic regions exhibited genomic imbalances in the analyzed 4 cell lines. The details of found CNV are outlined in Tables 4 and 5.

4. Discussion

Here 2 original cell lines and their corresponding NPC derivatives were studied by M-FISH and aCGH. It could be shown that the combination of molecular cytogenetics and aCGH leads to the detection of genetic imbalances on different levels of resolution.

M-FISH revealed normal karyotypes for the iPSC cell line and its derivative but a mosaic trisomy 20 in the NPC derived from ESC. Interestingly this was not detectable by aCGH. The latter can be due to the fact that the trisomy 20 was present in less than 50% of the cells. In our hands mosaics below 50% were not detectable yet using standard aCGH-software evaluation. No trisomy 20 was yet reported in any ESC or iPSC, only a 20q deletion was previously seen in one pluripotent stem cell line (Hollings et al., 1994).

Table 4
aCGH results of ESC cell line and its NPC derivative obtained from a healthy subject.

Chromosome	Cytoband	Begin	End	Gain [MB]	Loss [MB]
ESC					
1	q23.1	156,619,312	157,197,596	n.a.	0.578
7	p22.3–p22.2	289,912	2,705,543	2.416	n.a.
11	p15.5	357,497	2,002,404	1.645	n.a.
17	q25.3	76,890,993	77,084,795	1.938	n.a.
22	q11.21	18,471,124	19,063,627	0.593	n.a.
22	q11.23	22,677,759	22,720,395	n.a.	0.653
NPC from ESC					
1	q23.1	156,619,312	157,197,596	n.a.	0.578
11	p15.5	357,497	2,002,404	1.645	n.a.
15	q11.2	19,382,327	20,060,261	n.a.	0.678

Table 5
aCGH results of iPSC cell line and its NPC derivative obtained from a schizophrenic subject.

Chromosome	Cytoband	Begin	End	Gain [MB]	Loss [MB]
iPSC					
5	q23.1	118,913,710	118,980,301	n.a.	0.067
6	q22.1	116,871,473	116,981,694	n.a.	0.110
14	q32.32	102,446,974	102,499,423	0.052	n.a.
NPC from iPSC					
7	p22.3-p22.2	289,912	2,705,543	2.416	n.a.
14	q32.32	102,446,974	102,499,423	0.052	n.a.
17	q25.3	76,890,993	77,084,795	1.938	n.a.
20	q11.1–q11.21	28,265,913	30,611,064	2.345	n.a.

A general chromosomal instability in all studied lines (Rosler et al., 2004) could be suggested according to the results presented in Tables 1–3. However, this "instability" was most likely due to preparation artifacts as different, non-clonal chromosomes were lost in 54–64% of the evaluable 17 to 39 metaphases; the latter were selected from 100 to 200 metaphases being too clumsy and/or overspread.

aCGH proved the presence of CNVs in ESC and iPSC and their derived NPC. Our data support the view of two different origins for the structural variations as suggested by Martins-Taylor and Xu (2012): a) CNVs inherited from the original pluripotent cell source, either ESC or iPSC; and b) CNVs observed after the differentiation procedure and identified only in derived NPC. These observations indicate that differentiation ought to be considered in the same breadth as other types of manipulation (chiefly reprogramming and expansion of pluripotent cell lines) that are known to cause CNVs. The implications of this in the context of the potential development of cell therapies based on the differentiation of pluripotent cells cannot be underestimated (Stephenson et al., 2010).

Gains of copy numbers in 20q11.1–q11.21 as detected in one of the cell lines analyzed was already previously reported as a recurrent imbalance in stem cells (Spits et al., 2008; Elliott et al., 2010; Martins-Taylor et al., 2011). It was speculated, that this CNV leads to a growth advantage of the cells acquiring this CNV, as the gene BCL2L1 (OMIM *600039) having anti-apoptotic potential is included in this region (International Stem Cell Initiative, 2011). Interestingly in this study this amplification was present in the derivative NPC and not in the original iPSC, supporting the idea of acquisition of this CNV due to survival advantages under long term cultural conditions. CNVs classified as unique to IPSC, such as 1q31.3, 8q24.3 or 17q21.1 (Martins-Taylor et al., 2011), were not identified in this study, which could be attributed to the small number of samples analyzed.

Overall, routine cytogenetic analysis is severely limited in its capacity to detect many of those changes, which may have clinical implications. The synergistic use of M-FISH and aCGH analyses would cover most genomic structural abnormalities at different levels.

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