Recurrent Duplications of the Long Arm of Chromosome 1 in Human Pluripotent Stem Cell Lines

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Introduction

Gain of the (long) q-arm of chromosome 1 is a known recurrent abnormality in human pluripotent stem cells (hPSC) *in vitro*[1,2,3]. We catalogued the karyotype results of 50 embryonic stem cell (ESC) specimens and 103 induced pluripotent stem cell (iPSC) specimens that contained gain of 1q . The data from these 153 hPSC specimens were analyzed with the goal of finding the minimal overlapping region of the 1q abnormalities. Identifying this region provides a target for a rapid screening assay such as fluorescence *in situ* hybridization (FISH).

Methods and Materials

For G-banded karyotyping: Cell harvest, slide making, and staining techniques were all performed in accordance with standard cytogenetic protocols adapted for use with hPSC. For microarray analysis: High quality genomic DNA was extracted from a frozen cell pellet using the Qiagen DNeasy Blood and Tissue kit. DNA was labeled, amplified and hybridized onto a SurePrint G3 Human CGH+SNP Microarray Kit, 4x180k (NCBI Build 37/hg19) chip following Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis (v7.3). Data was analyzed with Agilent CytoGenomics 3.0.4.1. Fluorescence *in situ* hybridization (FISH) using the Kreatech FISH probe MDM4 (1q32) / Satellite Enumeration (SE) 1 was performed according to the manufacturer's protocol and visualized in Applied Spectral Imaging FISHView.

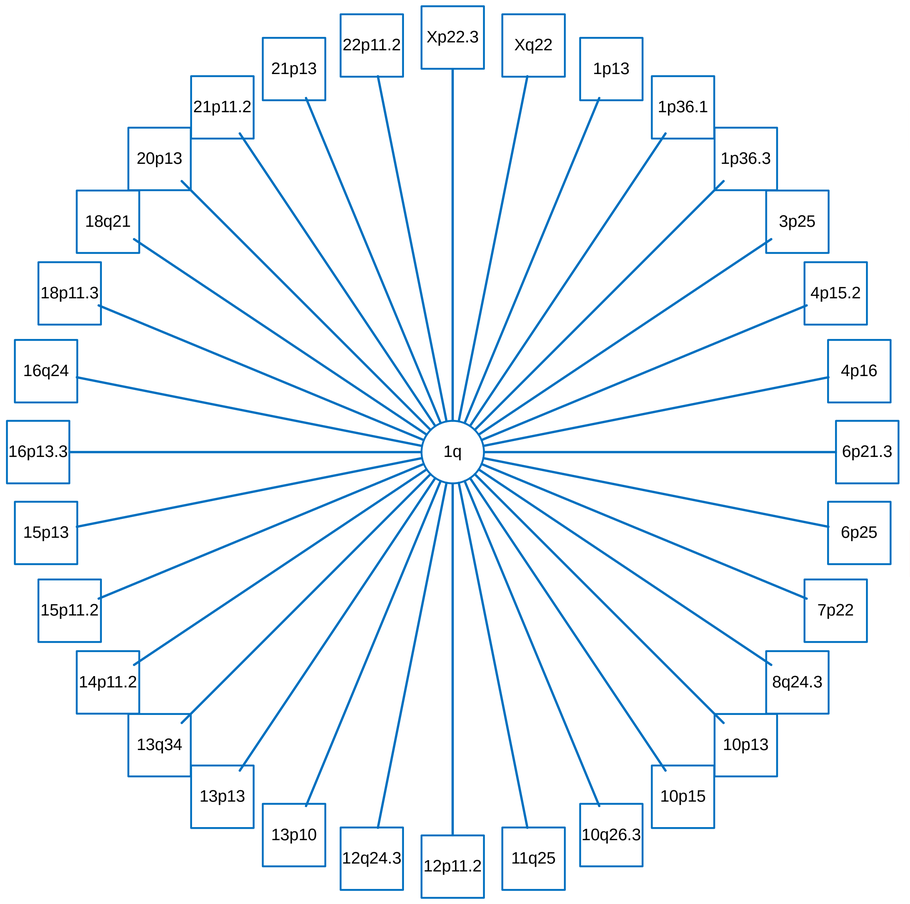

Figure 1. Translocation partners of 1q. We determined from the karyotype results of 153 hPSC specimens that 1q formed an unbalanced translocation with 32 different partners, an atypically large number (not previously reported).

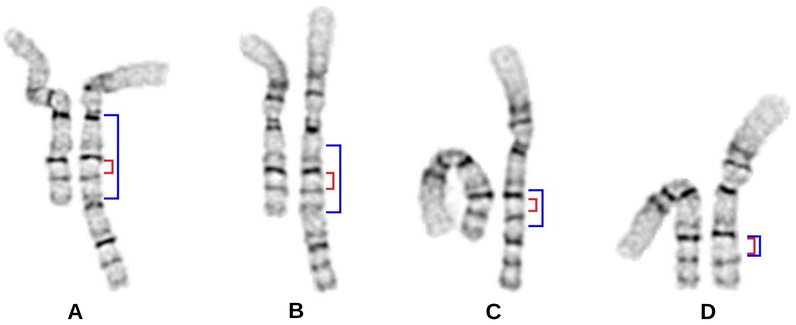

Figure 2. Four different examples of 1q duplications shown by G-banded karyotyping. The 1q gain is outlined in blue while the minimal overlapping region is outlined in red. **(A)** dup(1)(q12q44) **(B)** dup(1)(q23.1q44) **(C)** dup(1)(q25q42) **(D)** dup(1)(q32q32)

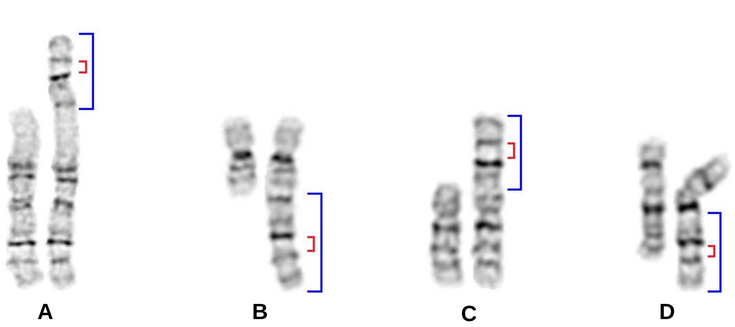

Figure 3. Four different examples of unbalanced translocations shown by G-banded karyotyping. The 1q gain is outlined in blue while the minimal overlapping region is outlined in red. **(A)** der(1)t(1;1)(p36.3;q11) **(B)** der(16)t(1;16)(q12;q24) **(C)** der(10)t(1;10)(q21;p13) **(D)** der(X)t(X;1)(q22;q21)

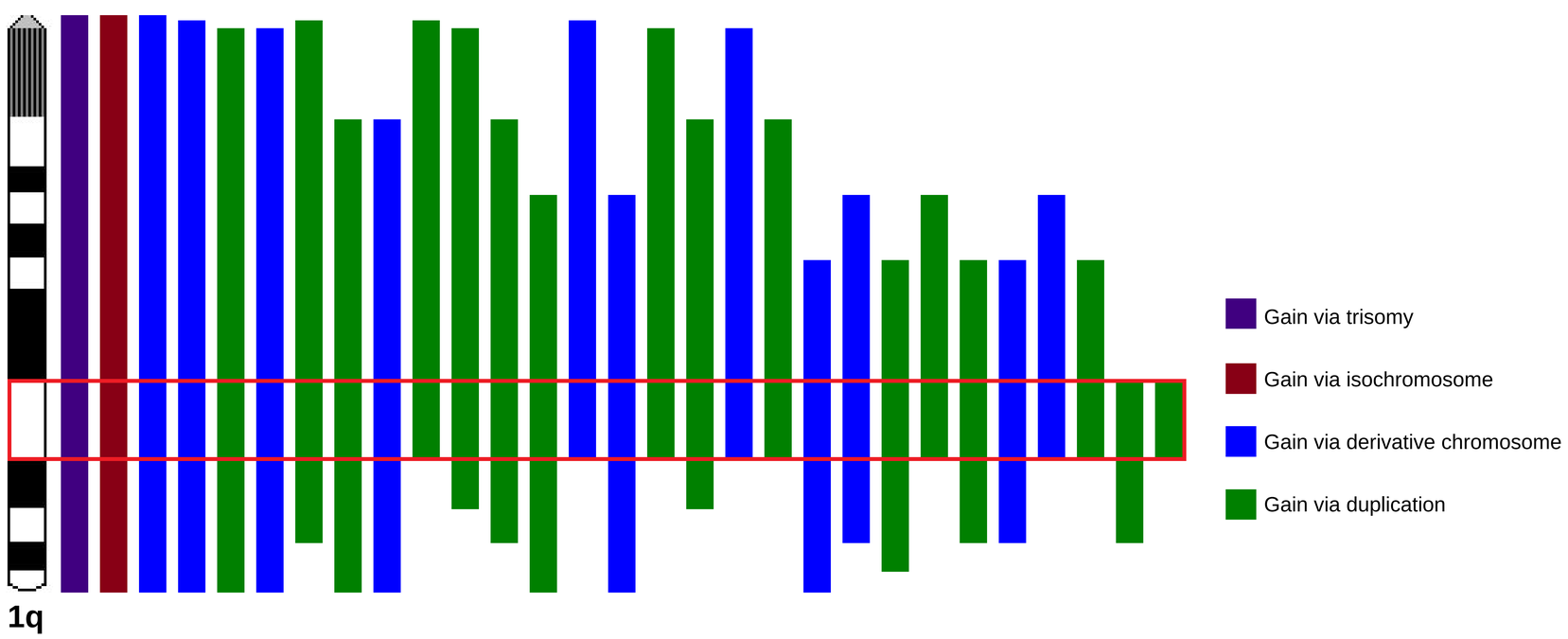

Figure 4. Ideogram representing distinct gains of 1q observed in the karyotype results of 153 hPSC specimens. Gains are color-coded according to the abnormality type and organized from largest to smallest. The 1q32 cytoband is outlined by a red border; all observed gains contain the 1q32 cytoband making it the minimal overlapping region.

Results

The karyotype results of 153 hPSC specimens contained a total of 159 abnormalities that resulted in gain of 1q, which occurred independent of both culture system (including suspension and adherent, feeder and feeder-free) and passage number (3 to 142). There was a striking diversity of 74 unique abnormalities and 32 different translocation partners for 1q (Figure 1). As varied as gains of 1q have shown to be, the 1q32 cytoband was present in every observation (Figures 2,3,4) as the minimal overlapping region. This finding was confirmed by microarray analysis (Figure 5) and the duplicated region was refined to chromosome 1 q32.1-q32.2, genomic position 201,815,320-207,635,067 (5,820kb). Using this data we selected a Kreatech FISH probe with the capability to detect every observed 1q gain through interphase FISH (Figure 6). Gains of 1q could confer an advantage in culture[3,4] and these findings may provide further insight into the mechanism(s) behind such enhancement.

References

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3. International Stem Cell Initiative. "Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage." *Nature biotechnology* 29.12 (2011): 1132-1144.
4. Dekel-Naftali, Michal, et al. "Screening of human pluripotent stem cells using CGH and FISH reveals low-grade mosaic aneuploidy and a recurrent amplification of chromosome 1q." *European Journal of Human Genetics* 20.12 (2012): 1248-1255.

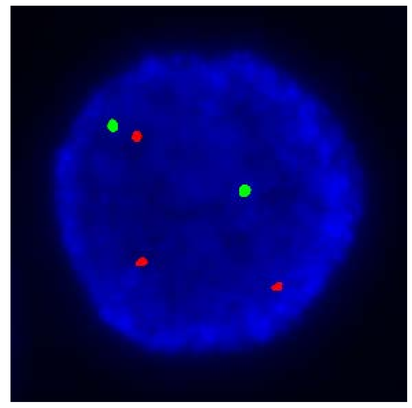

Figure 6. Fluorescence *in situ* hybridization (FISH) using the Kreatech FISH probe MDM4 (1q32) / Satellite Enumeration (SE) 1 dual-color assay. The MDM4 gene region probe is labeled red and the SE 1 probe is labeled green. This 3R2G signal pattern demonstrates gain of MDM4 (1q32).

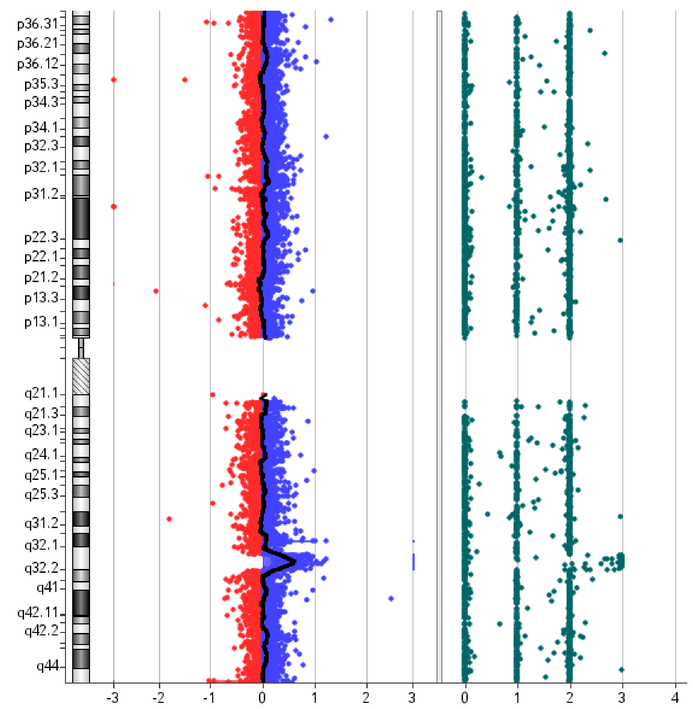

Figure 5. Agilent CGH+SNP microarray data demonstrating the smallest overlapping gain (genomic position 201,815,320-207,635,067 (5,820kb)) of 1q32.1-q32.2. Amplification of this region occurs in all 153 specimens.