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Human embryonic stem cells reveal recurrent genomic instability at 20q11.21

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By analyzing five human embryonic stem (hES) cell lines over long-term culture, we identified a recurrent genomic instability in the human genome. An amplification of 2.5–4.6 Mb at 20q11.21, encompassing ~23 genes in common, was detected in four cell lines of different origins. This amplification, which has been associated with oncogenic transformation, may provide a selective advantage to hES cells in culture.

Karyotypic abnormalities in hES cell lines, in particular gain of chromosomes 12, 17q or X, have been reported by many laboratories[1–3]. These defects were detected in conventional Giemsa-stained karyotypes, and the International Stem Cell Initiative has made Giemsa staining a required control for genomic integrity[4]. Smaller sequence changes have also been reported using screening techniques with higher resolution[1]. We have identified a genomic amplification of 20q11.21 in four independent hES cell lines (**Table 1**) using IntegraChip bacterial artificial chromosome (BAC) array-based comparative genomic hybridization (BAC aCGH), the Illumina high-density oligonucleotide array–based single nucleotide polymorphism (SNP) analysis and fluorescence *in situ* hybridization (FISH) (**Supplementary Methods** online). Our data show that in three of the four lines (SA01, H9 and VUB05-HD), the 20q11.21 duplication arose during long-term culture in our laboratory.

We cultured the hES cell lines SA01, H9, H1, VUB01 and VUB05-HD for a variable number of passages (**Supplementary Methods**). These lines had previously been shown to have a normal diploid karyotype (http://stemcells.nih.gov/research/registry). Manual dissection rather than an enzymatic method was systematically used to passage these lines.

BAC aCGH analyses revealed an amplification at 20q11.21 in SA01 at passage 83 (**Fig. 1a**), in H1 at passage 41 and in VUB05-HD at passage 103 (**Supplementary Fig. 1** online). This amplification was not found at earlier passages 30–41 in SA01 or passages 39–40 in VUB05-HD, indicating that it was acquired during long-term culture (**Table 1**). In the case of H1, none of our BAC aCGH or FISH experiments showed an absence of this aberration, so it is unclear when it arose. BAC aCGH analysis did not detect a 20q11.21 amplification in H9 (passages 34–74) or VUB01 (passages 85–150). We further investigated the genomic integrity of SA01 at passage 49 and of VUB05-HD at passage 94 using the Illumina high-density

Table 1 20q11.21 amplification detection

Lines	Source	Batch	Passage number	Technology	Gain of 20q11.21[a]	Other karyotypic abnormalities
SA01	Cellartis	a	P30	aCGH	–	No
		a	P41	aCGH	–	No
		b	P21	FISH	–	nt
		b	P24	FISH	–	nt
		b	P32	aCGH	–	No
		b	P37	FISH	–	nt
		b	P49	SNP	Yes	No
		b	P56	FISH	Yes	nt
		c	P83	aCGH	Yes	No
VUB01	AZ-VUB	a	P80	FISH	–	nt
		a	P85	aCGH	–	No
		a	P94	aCGH	–	No
		a	P126	FISH	–	nt
		b	P150	aCGH	–	Trisomy 12
VUB05-HD	AZ-VUB	a	P34	FISH	–	nt
		a	P39	aCGH	–	Trisomy 12
		b	P40	aCGH	–	Trisomy 12
		c	P94	SNP	–	No
		c	P98	FISH	–	nt
		c	P103	aCGH	Yes	No
		c	P105	FISH	Yes	nt
H9	WiCell	a	P34	aCGH	–	No
		a	P53	FISH	–	nt
		a	P55	FISH	–	nt
		a	P74	aCGH	–	No
		a	P76	FISH	Yes	nt
H1	WiCell	a	P24	FISH	Yes	nt
		a	P41	aCGH	Yes	No
		a	P53	aCGH	Yes	No
		a	P64	FISH	Yes[b]	nt

[a]"–" no gain of 20q11.21 detected by molecular karyotype (aCGH or SNP) or less than 1.5% of cells with three signals detected by FISH analyses. [b]Ins(1;20)(p36.3;q11.21). nt, not tested.

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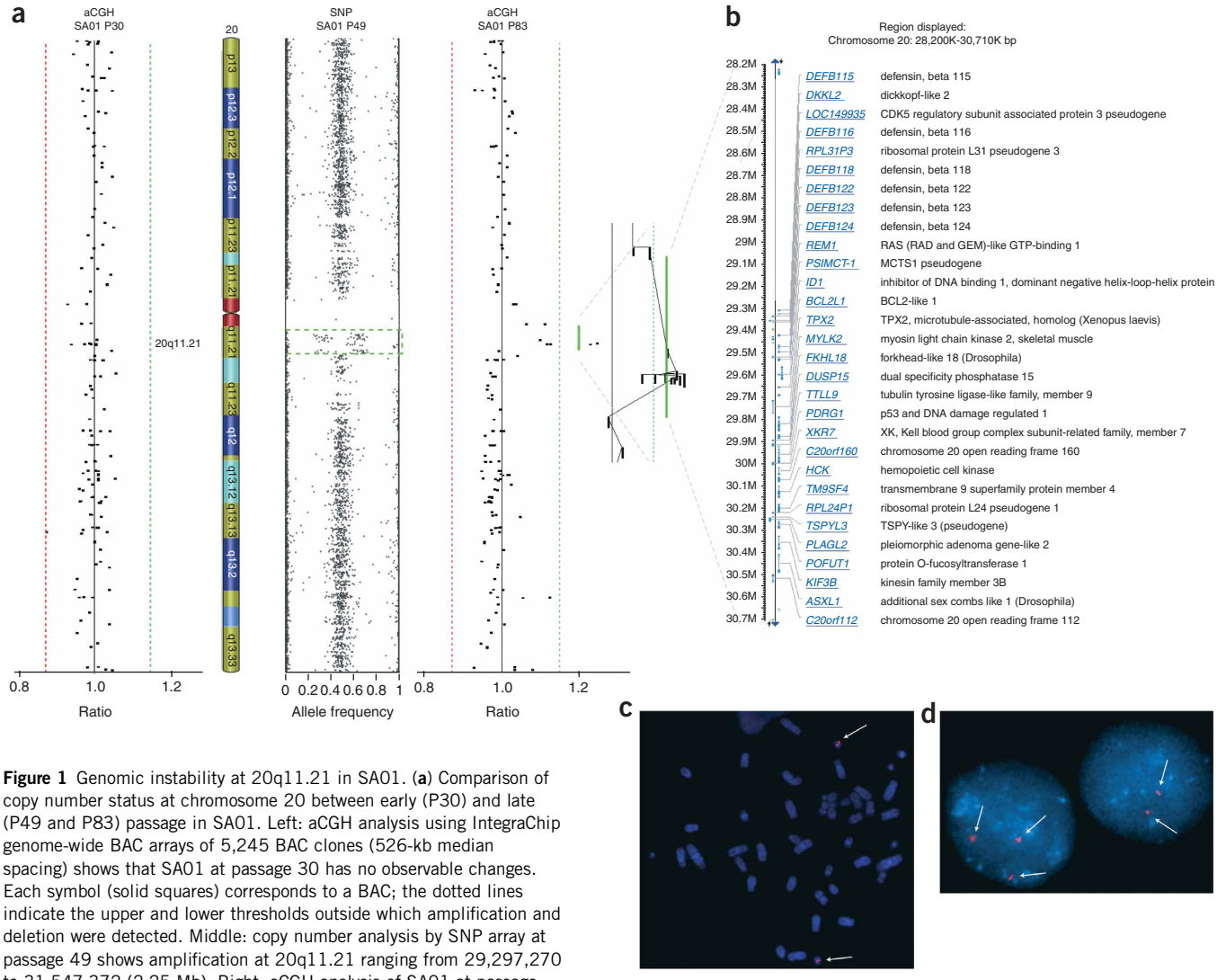

Figure 1 Genomic instability at 20q11.21 in SA01. (**a**) Comparison of copy number status at chromosome 20 between early (P30) and late (P49 and P83) passage in SA01. Left: aCGH analysis using IntegraChip genome-wide BAC arrays of 5,245 BAC clones (526-kb median spacing) shows that SA01 at passage 30 has no observable changes. Each symbol (solid squares) corresponds to a BAC; the dotted lines indicate the upper and lower thresholds outside which amplification and deletion were detected. Middle: copy number analysis by SNP array at passage 49 shows amplification at 20q11.21 ranging from 29,297,270 to 31,547,372 (2.25 Mb). Right: aCGH analysis of SA01 at passage 83 shows amplification at 20q11.21 ranging from 28,205,000 to 30,711,000 (2.5 Mb, genome build 36). (**b**) The 20q11.21 locus is depicted with increasing resolution from left to right, with genes within the amplified region. Some of these genes may provide a selective advantage to hES cells in culture (adapted from NCBI Mapviewer). (**c**,**d**) FISH analysis on metaphases (SA01) showing two signals (**c**, arrows) at 20q11.21, whereas three signals (**d**, arrows) in 83/150 interphase nuclei were observed, corresponding to a duplication of the 20q11.21 region on chromosome 20.

oligonucleotide array–based SNP analysis and confirmed the presence of the 20q11.21 amplification in SA01 (**Fig. 1a**,**b**) but not in VUB05-HD. HES cell lines with the 20q11.21 amplification were not noticeably different from those without genomic alteration with respect to growth, morphology and the expression of canonical hES cell markers.

To estimate the proportion of cells affected during continued cell passage, we used FISH with BACs specific to the 20q11.21 region. In SA01, amplification of 20q11.21 was not seen at passages 21, 24 and 37 and was detected in 55% of the cells at passage 56 (**Fig. 1c**,**d**). In H9, the amplification was not found at passages 53 and 55 and was detected in 25% of the cells at passage 76 (whereas, using BAC aCGH, the amplification was not seen in this line up to passage 74). In H1, the amplification was detected in 24% and 47% of the cells at passages 24 and 64, respectively (**Supplementary Fig. 2** and **Supplementary Table 1** online). In VUB01, no amplification was seen at passages 80 and 126. Finally, in VUB05-HD, the amplification was not seen at passages 34 and 98 and was detected in 57% of the cells at passage 105. The extra copy of the 20q11.21 region was found inserted either at the 1p36.3 region (H1) or as a tandem or inverted repeat at 20q11.21 (SA01, H9, VUB05-HD).

Therefore, four of the five hES cell lines grown in our laboratory were found by at least one technique to have an amplified region localized to chromosome 20q11.21, ranging in size from 2.5 Mb to 4.6 Mb. It is unlikely that this amplification was due to a technical artifact related to culturing technique specific to our laboratory as one cell line (VUB01) cultured similarly did not show such an alteration. Neither is this genomic instability cell line–dependent as the same amplification has been observed by other authors in three other hES cell lines: VUB07 (C. Spits *et al.* 6th International Society for Stem Cell Research Meeting abstract), H7 (ref. 1) and HSF1 (ref. 5), although in the latter two cases it was considered a mutation[1] or a copy number variant[5] in the absence of data for the same cell lines at different passage numbers. Systematic analysis of several hES cell lines over long-term culture has thus allowed us to identify a region of genomic instability in the human genome. The amplified region encompasses about 23 genes (**Fig. 1b**) and a microRNA named hsa-miR-1825

located within *POFUT1*. This region corresponds to the mouse 2H region—syntenic to human 20q11-13—which has been considered 'hypermutable', as it is frequently involved in chromosomal rearrangements during the early stages of myeloid leukemia induction following ionizing radiation[6]. The high instability of microsatellite D2MIT140 marker, mapped to mouse chromosome 2H, is hypothesized to play a role in this phenomenon[7]. The 20q11.21 region is amplified in breast carcinomas[8,9], lung cancer[10], melanoma[11], hepatocellular carcinoma[12], bladder cancer[13] and early-stage cervical cancer[14]. All these data suggest that this region contains genes whose amplification provides a proliferative advantage, both for cancer progression and for hES cell growth. Of the ~23 genes contained in the smallest amplified region (**Fig. 1b**), several have been implicated in cell survival (*BCL2L1*, *PDRG1*), cell growth (*ID1*) or the cell cycle (*TPX2*, *KIF3B*). The miRNA hsa-miR-1825, located in the 7th exon of *POFUT1*, may be another good candidate as miRNAs are considered a novel class of genes contributing to tumorigenesis[15]. SNP and CGH data have been deposited in the GEO database under the accession number GSE13565.

Note: Supplementary information is available on the Nature Biotechnology website.

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AUTHOR CONTRIBUTIONS

N.L., M.P. and A.L.P. designed the study. N.L. carried out and analyzed CGH and SNP experiments. C.B. carried out the FISH experiments. N.L. and M.F. did all hES cell culture, except for that of H1 cells, which were cultured by O.F. The FISH experiments were designed and analyzed by G.T., A.B.-G. and C.B. A.B.-G. and G.T. edited the paper. N.L., M.P. and A.L.P. contributed to writing the paper.

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1. Maitra, A. *et al. Nat. Genet.* **37**, 1099–1103 (2005).
2. Josephson, R. *et al. BMC Biol.* **4**, 28 (2006).
3. Baker, D.E. *et al. Nat. Biotechnol.* **25**, 207–215 (2007).
4. Loring, J.F. & Rao, M.S. *Stem Cells* **24**, 145–150 (2006).
5. Wu, H. *et al. Stem Cells* **26**, 1484–1489 (2008).
6. Rithidech, K., Bond, V.P., Cronkite, E.P., Thompson, M.H. & Bullis, J.E. *Proc. Natl. Acad. Sci. USA* **92**, 1152–1156 (1995).
7. Rithidech, K.N., Dunn, J.J., Gordon, C.R., Cronkite, E.P. & Bond, V.P. *Blood Cells Mol. Dis.* **23**, 99–109 (1997).
8. Tanner, M.M. *et al. Cancer Res.* **56**, 3441–3445 (1996).
9. Guan, X.Y. *et al. Cancer Res.* **56**, 3446–3450 (1996).
10. Tonon, G. *et al. Proc. Natl. Acad. Sci. USA* **102**, 9625–9630 (2005).
11. Koynova, D.K. *et al. Melanoma Res.* **17**, 37–41 (2007).
12. Midorikawa, Y. *et al. Oncogene* **25**, 5581–5590 (2006).
13. Hurst, C.D. *et al. Oncogene* **23**, 2250–2263 (2004).
14. Scotto, L. *et al. Genes Chromosom. Cancer* **47**, 755–765 (2008).
15. Calin, G.A. *et al. Proc. Natl. Acad. Sci. USA* **101**, 2999–3004 (2004).

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