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Large-Scale Analysis Reveals Acquisition of Lineage-Specific Chromosomal Aberrations in Human Adult Stem Cells

Uri Ben-David,[1] Yoav Mayshar,[1,*] and Nissim Benvenisty[1,*]
[1]Stem Cell Unit, Department of Genetics, Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, 91904, Israel
*Correspondence: myoavi@cc.huji.ac.il (Y.M.), nissimb@cc.huji.ac.il (N.B.)
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In this study, we assessed the genetic integrity of over 400 samples of human multipotent stem cells using gene expression data sets. Our analysis reveals that neural and mesenchymal stem cells acquire characteristic large chromosomal aberrations at a similar, or somewhat lower, frequency to that seen in pluripotent stem cells, sometimes within a few passages in culture. Some of the identified chromosomal abnormalities can also be detected in human tumors of the respective tissues.

Human embryonic stem cells (hESCs) acquire chromosomal aberrations in culture, in a process known as culture adaptation (Baker et al., 2007; Mayshar et al., 2010). These aberrations may increase the tumorigenicity of the cells (reviewed in Ben-David and Benvenisty, 2011) and disrupt their differentiation capacity (Harrison et al., 2007). While they are assumed to be acquired stochastically, specific aberrations provide selective advantage, and are thus detected at a much higher frequency than others. Recently, chromosomal aberrations were also documented in human induced pluripotent stem cells (hiPSCs) (Hussein et al., 2011; Laurent et al., 2011; Mayshar et al., 2010).

While chromosomal aberrations in pluripotent stem cells (PSCs) have been extensively studied and characterized (Baker et al., 2007; Hussein et al., 2011; Laurent et al., 2011; Mayshar et al., 2010), the phenomenon of culture adaptation in human multipotent stem cells is much less explored. Individual cases of chromosomal aberrations have been detected in both human neural stem cells (NSCs) (Sareen et al., 2009) and human mesenchymal stem cells (MSCs) (Buyanovskaya et al., 2009; Takeuchi et al., 2009), but no comprehensive study of the genomic stability of multipotent stem cells has been carried out to date. Furthermore, even in cases where the chromosomal integrity of human multipotent stem cells was documented, such analyses were mostly performed at early passages of their growth. Thus, multipotent stem cells, as opposed to PSCs, are generally considered to be genetically stable (Bernardo et al., 2007;

De Filippis et al., 2007; Meza-Zepeda et al., 2008; Villa et al., 2004), and are currently used in clinical trials in humans.

We recently developed a method for detecting chromosomal aberrations in human PSCs, based on the gene expression patterns of these cell lines (Mayshar et al., 2010). Here, we expanded our analysis of human PSCs, and in addition applied the same methodology to carry out a comprehensive analysis of large chromosomal aberrations in 144 samples of MSCs, 97 samples of NSCs, and 177 samples of hematopoietic stem/progenitor cells (HSPCs), from 45 independent studies (Figure 1A). We focused on the analysis of the genomic integrity of whole chromosomes or chromosome arms. Only aberrations that met the stringent criteria for statistical significance in both of the bioinformatic tools applied are presented and discussed (see Supplemental Experimental Procedures).
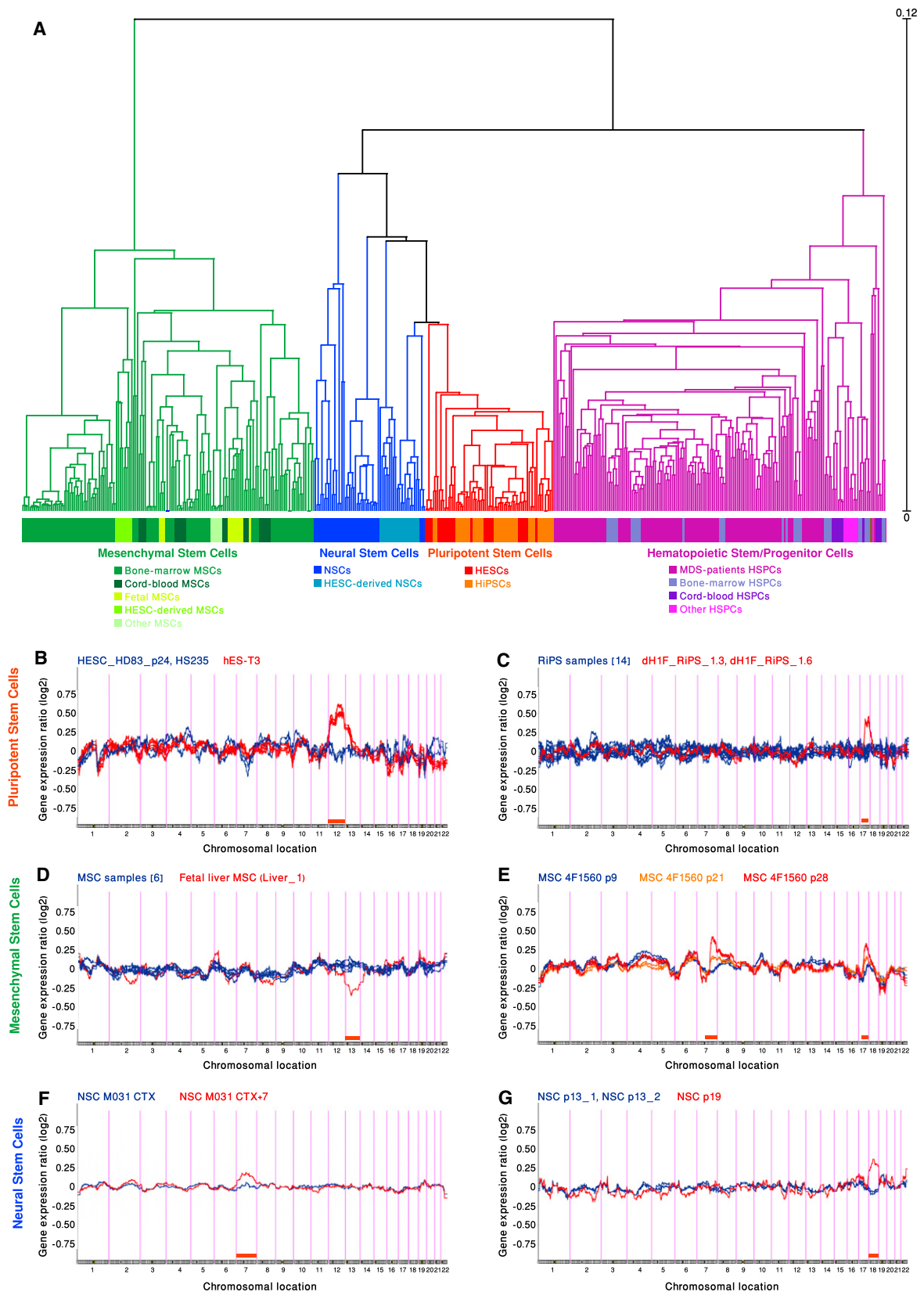
Analysis of Large Chromosomal Aberrations in PSCs

We initially completed a comprehensive analysis of chromosomal aberrations in PSCs. Formerly, we identified three sources for chromosomal aberrations in hiPSCs: aberrations of somatic origin, aberrations that occur during reprogramming, and aberrations acquired in culture (Mayshar et al., 2010). Here, we greatly expanded our published analysis of the PSC data sets, studying 39 additional hESC samples and 65 additional hiPSC samples from 13 recent studies (see Table S1). Although the most common autosomal aberrations in hESCs are tri-

somies 12 and 17 (Baker et al., 2007; Mayshar et al., 2010), previous studies failed to detect trisomy 17 in hiPSCs, and suggested this might be a difference between these cell types (Mayshar et al., 2010; Taapken et al., 2011). In the current analysis of PSCs, we detected further cases of previously described aberrations (Figure 1B), and identified trisomy 17 in hiPSCs in cell lines generated through reprogramming with synthetic mRNA molecules (Figure 1C). Because this trisomy was not identified in the parental somatic cell line (Warren et al., 2010), and because it appeared in culture at an early passage of the hiPSCs, it appears to be an example of a genomic aberration arising through selective pressure during or immediately following the reprogramming process. This finding also supports the notion that hiPSCs are prone to chromosomal aberrations regardless of the reprogramming method used (Ben-David and Benvenisty, 2011; Ben-David et al., 2010), because we previously identified aberrations in cell lines reprogrammed with integrating viruses, episomal vectors, and recombinant proteins. An ideogram of the chromosomal aberrations identified in PSCs is presented as Figure 2A, and a full analysis of the aberrations detected can be found in Table S1. Large chromosomal aberrations were identified in ~9% of all the samples analyzed, consistent with our previous report (Mayshar et al., 2010).

Analysis of Large Chromosomal Aberrations in Adult Stem Cells
Chromosomal aberrations are known to accumulate in various cell types in culture.

However, their accumulation in cultures of adult stem cells is still a matter of open debate. Although previous studies have identified chromosomal aberrations in cultured human MSCs (Røsland et al., 2009; Ueyama et al., 2011), these reports analyzed a rather small number of MSC lines from bone marrow origin only, and were thus limited in their ability to detect recurrent chromosomal aberrations in various types of MSCs. Other studies reported that human MSCs retained chromosomal stability following long-term culture in vitro (Bernardo et al., 2007; Zhang et al., 2007). Here we analyzed published expression profiles for MSCs from five distinct origins. The MSC data set consisted of 135 human MSC samples and 9 hESC-derived MSCs, all from 22 independent studies. Because human MSCs from various sources (such as bone marrow, adipose, and umbilical cord) clustered together with each other and with hESC-derived MSCs in an unsupervised hierarchical clustering (Figure 1A), all the MSC types could be analyzed using a single common baseline. The analysis detected two monosomies of chromosome 13 (from two independent studies), as well as four monosomies of chromosome 6q (from two independent studies) (Figures 1D, 2B, and S1A). None of these aberrations was reported in the respective original study. Our analysis also identified one line that acquired gains of chromosomes 7q and 17q (Figure 1E) and one line that acquired trisomy 19 (Figure S1B). The latter aberrations have not been previously identified in MSCs. An ideogram of the chromosomal aberrations identified in MSCs is presented as Figure 2B, and a full analysis of the detected aberrations can be found in Table S1. Overall, we report a frequency of aberrations of ~4% for MSCs, which could possibly

account for the failure of previous smaller studies to detect them.

The NSC data set comprised 58 human NSC samples and 39 hESC-derived NSC samples, all from 11 independent studies. The analysis identified a trisomy of chromosome 7 (Figure 1F) and recurrent cases of trisomy 19 (Figures 2C and S1C), aberrations that have been previously described to occur in NSCs (Sareen et al., 2009). One of the detected aberrations had been reported in the original study (Sareen et al., 2009), while the others had not. The analysis of the NSCs also revealed a trisomy and a monosomy of chromosome 18 (Figures 1G and 2C) and a trisomy of chromosome 10 (Figure 2C), which had not been previously reported in NSCs. The analysis of the hESC-derived NSCs also revealed a trisomy of chromosome 20q, which had not been previously identified in NSCs (Figure 2c). Because trisomy 20q is a common aberration in hESCs (Lefort et al., 2008; Spits et al., 2008), this aberration probably arose prior to the differentiation of the hESCs. Thus, not surprisingly, hESC-derived NSCs seem to be susceptible to both the typical PSC aberrations and the typical NSC aberrations. The nature of the acquired aberrations in each cell line would probably depend on the time it spent in culture in each of these states. An ideogram of the chromosomal aberrations identified in NSCs is presented as Figure 2C, and a full analysis of the detected aberrations can be found in Table S1. Overall, we report a frequency of aberrations of ~9% for NSCs, a similar frequency to the one observed for PSCs.

Finally, we analyzed a data set of 177 CD34+ HSPC samples from 12 independent studies. Of these, 55 samples were from healthy individuals, and 122 were from karyotyped myeloid dysplasia syn-

drome (MDS) patients that we could use as controls (Pellagatti et al., 2010). Because CD34+ samples from various sources (bone marrow, peripheral blood, and umbilical cord blood) clustered together with each other and with samples from MDS patients in an unsupervised hierarchical clustering (Figure 1A), all the HSPC types could be readily analyzed using a single common baseline. Our analysis correctly detected 34 out of the 36 reported aberrations in the patient-derived HSPCs (the remaining two were identified by only one of the bioinformatic tests). A full analysis of the detected aberrations can be found in Table S1, and an example is shown in Figure S1D. In contrast to the other stem cells analyzed, and to the HSPCs derived from MDS patients, we could not detect any aberration in the 55 samples of healthy donor-derived HSPCs. Unlike NSCs and MSCs, HSPCs are not routinely propagated in vitro for multiple passages. Hence, the absence of aberrations in these cells does not necessarily suggest that they are less susceptible to chromosomal aberrations in comparison with other multipotent stem cells; rather, it suggests that adult stem cells are generally euploid in vivo, and may acquire large chromosomal aberrations upon in vitro adaptation to culture.

The Frequency, Rapidity, and Specificity of the Acquisition of Chromosomal Aberrations

A comparison of the frequency of chromosomal aberrations in the different types of stem cells reveals a rather similar frequency of aberrations in all the stem cell types that are propagated in culture: 24 aberrations in 19 out of 208 samples (~9%) in PSCs, 9 aberrations in 9 out of 97 samples (~9%) in NSCs, and

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Figure 1. Gene Expression Patterns Reveal Chromosomal Aberrations in Human Stem Cells

(A) Unsupervised hierarchical clustering of the MSCs (green branches), NSCs (blue branches), HSPCs (purple branches), and PSCs (red branches) analyzed in the current study (Affymetrix HG-U133plus2 platform). The distinct groups of stem cells cluster apart from each other, and were thus analyzed separately. The various origins of the samples inside each stem cell group are color-coded.

(B–G) Moving average plots of gene expression levels along the whole genome of PSCs (B and C), MSCs (D and E), and NSCs (F and G). (B) Six samples of the hESC line, hES-T3, cultured in various conditions, demonstrate trisomy of chromosome 12 in this cell line (red lines, $p = 5 \times e^{-39}$). Two normal hESC lines from different studies (hESC_HD83_p24 and hESC HS235) are presented as controls (blue lines). (C) Synthetic mRNA-induced hiPSC lines, dH1F_RiPS_1.3 and dH1F_RiPS_1.6, demonstrate trisomy of 17q (red lines, $p = 6 \times e^{-26}$). Fourteen other samples from the same study are presented as controls (blue lines). (D) Fetal liver-derived MSC line, Liver_1, demonstrates monosomy 13 (red line, $p = 3 \times e^{-10}$). Six other MSC samples from the same study are presented as controls (blue lines). (E) Bone marrow-derived MSC line, #4F1560, acquired trisomies of 7q and 17q during its passaging in culture. The cells were normal at passage 9 (three replicates, blue lines), the trisomy is already evident at passage 21 (two replicates, orange lines), and it took over the culture by passage 28 (three replicates, red lines, $p = 2 \times e^{-7}$ and $p = 1 \times e^{-7}$ for trisomies 7q and 17q, respectively). (F) Fetal cortex-derived NSC line, M031 CTX +7, demonstrates trisomy 7 (red line, $p = 2 \times e^{-8}$). This trisomy was previously identified in this cell line. The diploid NSC line from the same study, M031 CTX, is presented as control (blue line). (G) Fetal-derived NSC line acquired trisomy 18 during its passaging in culture. The cells were normal at passage 9 (two replicates, blue lines), but acquired the trisomy by passage 19 (red line, $p = 1 \times e^{-9}$).
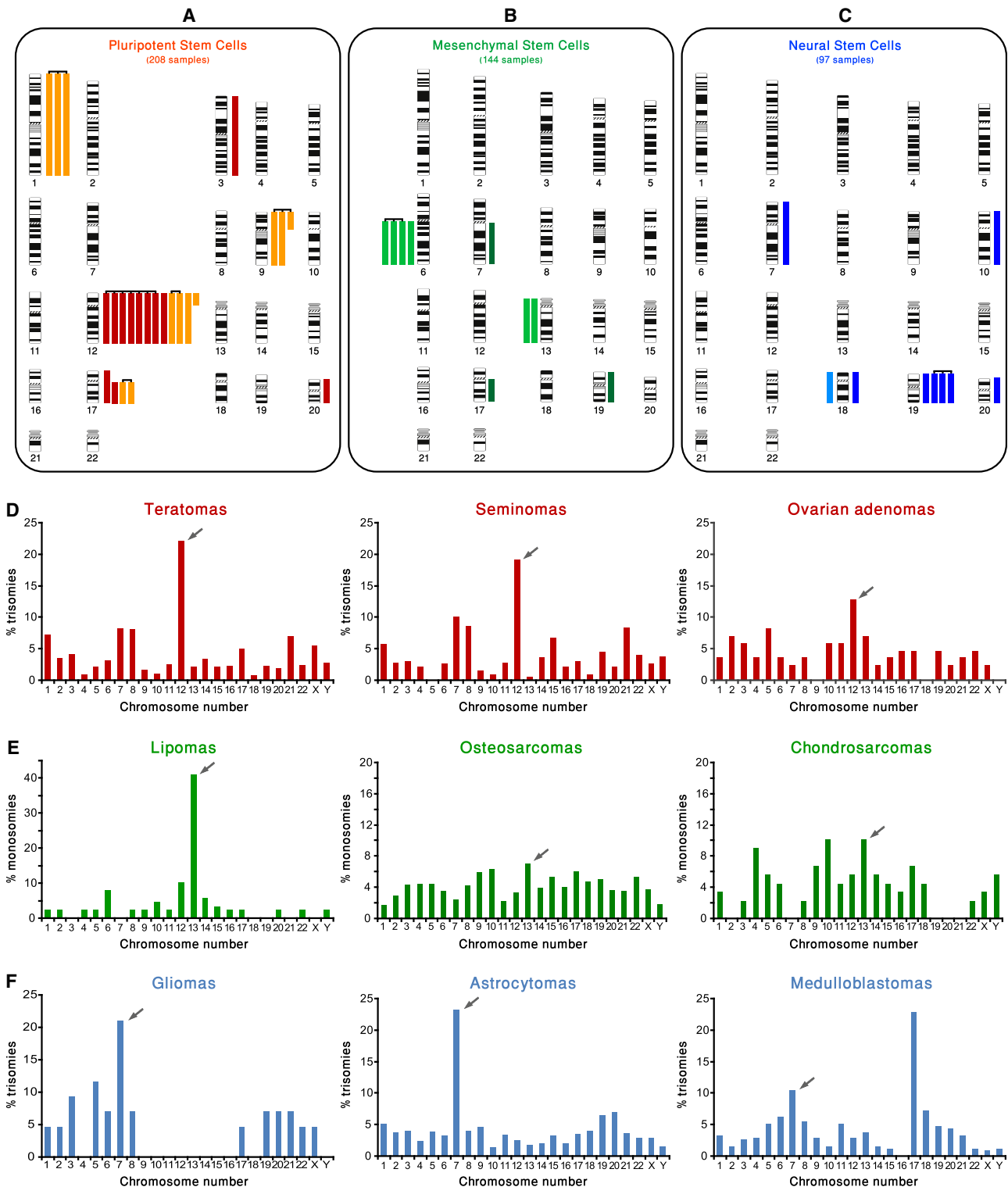
See also Figure S1 and Table S1.

Figure 2. Different Types of Stem Cells Acquire Distinct Chromosomal Aberrations
(A–C) Ideograms representing the chromosomal aberrations identified in (A) PSCs, (B) MSCs, and (C) NSCs. Bars to the right of the chromosome represent gains, and bars to the left of the chromosome represent deletions. In the ideogram of PSCs (A), red and orange represent hESCs and hiPSCs, respectively. The PSC aberrations identified in the current study are shown together with the aberrations previously identified by Mayshar et al. (2010). Chromosomal aberrations in samples from similar cells from the same study are interconnected by a line, and were considered as a single aberration for the purpose of statistical analysis. (D–F) Some of the recurrent aberrations detected in stem cells are the most common aberrations in tumors of the same tissue origin. The frequency of chromosomal aberrations in various types of tumors was calculated using the National Cancer Institute "Recurrent Chromosomal Aberrations in Cancer Database

9 aberrations in 6 out of 144 samples (∼4%) in MSCs. Thus, based on the data set we have analyzed, it seems that NSCs acquire large chromosomal aberrations at a similar frequency to that seen in PSCs, while MSCs acquire large chromosomal aberrations at a somewhat lower frequency.

The chromosomal aberrations are not uniformly distributed among the chromosomes (p = 0.043, Chi-square goodness-of-fit test), and the specific aberrations a cell line is likely to acquire depend on the stem cell group to which it belongs (p = 0.007, Fisher's exact test). While PSCs and NSCs tend to acquire trisomies (100% and 89% of the aberrations, respectively), MSCs tend to acquire monosomies (∼67% of the aberrations, p = 0.012, Fisher's exact test), in line with previous reports (Buyanovskaya et al., 2009). Most of the identified aberrations are recurrent in a specific cell type, such as trisomy 12 for PSCs, monosomies 6q and 13 for MSCs, and trisomy 19 for NSCs. Moreover, multipotent stem cells that are derived from PCSs also harbor the risk of acquiring the typical chromosomal aberrations of PSCs (such as trisomy 20), probably during their pluripotent stage in culture. Taken together, these data demonstrate that chromosomal aberrations are a common feature of stem cells propagated in vitro, and further suggest that each type of stem cell is prone to acquire a unique set of chromosomal aberrations (illustrated in Figure S2).

It is important to stress that the frequencies of the aberrations identified in this study refer only to aberrations that encompass whole chromosomes or chromosome arms, and thus constitute an underestimation of the possible total number of genomic abnormalities in adult stem cells. Recent studies have revealed small copy number variations (CNVs) and coding mutations in hiPSCs, some of which were shown to exist in the fibroblasts of origin and were selected for during reprogramming, while others arose de novo during this process or after the growth of the cells in culture (Gore et al., 2011; Hussein et al., 2011; Laurent et al., 2011). It is therefore reasonable to assume that CNVs and point mutations would also arise during the culture propagation of multipotent stem cells.

We recently reported that trisomy 12 accumulates in PSC cultures rapidly, and could take over the culture within as few as five passages (Mayshar et al., 2010). In this study, two cell lines that acquired chromosomal aberrations in culture were also analyzed at earlier time points. Thus, we show that in MSCs chromosomal aberrations can take over the culture in as few as seven passages (Figure 1E), and in NSCs, in as few as six passages (Figure 1G). We conclude, therefore, that multipotent stem cells are prone to acquire advantageous chromosomal aberrations, which enable them to rapidly outgrow the normal cell population, at a similar rate as that previously reported for PSCs.

An Analysis of Lineage-Specific Chromosomal Aberrations In Vitro and In Vivo

We next looked at the relationship between the chromosomal aberrations we detected in stem cell cultures and those seen in human tumors. In order to perform an unbiased quantification of the aberrations in different types of tumors, we gathered data from thousands of tumors of the same tissues as the various stem cells analyzed, using a well-established database of chromosomal aberrations in cancer (Mitelman et al., 2007, 2011). A calculation of the frequency of chromosomal aberrations in these tumors revealed a partial correlation between the lineage-specific aberrations that arise in stem cell cultures and the ones most common in tumors of the respective tissue. The association is most prominent in PSCs: trisomy 12 is the most common aberration in PSC cultures, and is by far the most common aberration in three types of tumors of germ cell tissues (teratomas, seminomas, and ovarian adenomas) (Figure 2D). In MSCs, two aberrations were found to recur in independent studies; one of them, monosomy 13, is also strongly related to mesenchymal tumors, because it was found to be a frequent monosomy in bone and soft tissue tumors (lipomas, as well as chondrosarcomas and osteosarcomas) (Figure 2E). In NSCs, the association is weaker: trisomy 19 was found to arise recurrently, but trisomy 7 is the most prevalent in various brain tumors (gliomas, astrocytomas, and medulloblastomas) (Figure 2F). Trisomy 7 was reported to recur in NSCs (Sareen et al., 2009), but was identified only once in the current analysis.

The associations between chromosomal aberrations in stem cell cultures and in tumors of the same tissues do not necessarily mean that stem cells that acquired these aberrations would be more tumorigenic; they do imply, however, that specific aberrations at least confer growth advantage in a cell lineage-specific manner, both to stem cells in vitro and to tumors in vivo. If this were true, we would expect these two phenomena to share at least some of the genes involved. Supporting this notion, *Retinoblastoma* (*RB1*), which is located on 13q14, was found to be downregulated in many mesenchymal tumors with chromosome 13 deletion (Dahlén et al., 2003; Yamaguchi et al., 1996), and was also significantly downregulated in the two lines detected in our analysis to harbor monosomy 13 (2.2-fold and 5.3-fold decrease, p = 0.015, Student's t test).

Concluding Remarks

Transplantation of human adult stem cells may result in tumor formation (Amariglio et al., 2009; Casalbore et al., 2009). In the current analysis we identified stem cell-specific chromosomal aberrations, and compared the frequency, the identity, and the acquisition rate of these

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Searcher." (D) The relative frequency of trisomies, gains, and isochromosomes of each chromosome in three types of tumors of germ cell tissues. Trisomy 12 is the most common aberration in mature and immature teratomas (found in 182/827 cases), seminomas of the testis and the ovary (91/479), and adenomas of the ovary (11/87). (E) The relative frequency of monosomies and deletions of each chromosome in three types of tumors of mesenchymal tissues. Monosomy 13 is the most frequent aberration in lipomas (36/88), skeletal osteosarcomas (49/694), and dedifferentiated chondrosarcomas (9/89).
(F) The relative frequency of trisomies, gains, and isochromosomes in three types of neural tumors. Trisomy 7 is the most common aberration in gliomas (9/43) and astrocytomas (256/1107), and is also frequently found in medulloblastomas (29/280). Trisomy 17, which was not detected in the analysis of NSCs, is the most common aberration in medulloblastomas. Arrows indicate chromosomes that were identified in stem cell cultures of a specific type and are also frequent in tumors of the same tissue, revealing a possible correlation between the two (the correlation is most evident for PSCs and germ cell tumors, less significant for MSCs and mesenchymal tumors, and marginal for NSCs and neural tumors).
See also Figure S2 and Table S1.

aberrations between different types of stem cells. We found that (1) NSCs and MSCs acquire large chromosomal aberrations in culture at a similar (for NSCs) or somewhat lower (for MSCs) frequency to that seen in PSCs; (2) different stem cell types acquire distinct chromosomal abnormalities; and (3) once these aberrations occur, they take over the culture rapidly. Thus, we conclude that, as for pluripotent cells, the genomic stability of multipotent stem cells in culture should also be analyzed carefully and regularly. Large chromosomal aberrations might also arise in HSPCs, once the required conditions for their routine in vitro propagation are finally discovered. Chromosomal aberrations in PSCs have been previously suggested to increase their tumorigenicity (Ben-David et al., 2010); such aberrations might affect the safety of aberrant multipotent stem cells as well. Thus, validating the genomic integrity of stem cells of all types in culture is crucial, both for the correct interpretation of biological results and for their safe implementation in cell therapy.

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