World Journal of Stem Cells

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4252/wjsc.v8.i6.216

World J Stem Cells 2016 June 26; 8(6): 216-222
ISSN 1948-0210 (online)

MINIREVIEWS

Aneuploidy in stem cells

Jorge Garcia-Martinez, Bjorn Bakker, Klaske M Schukken, Judith E Simon, Floris Foijer

Jorge Garcia-Martinez, Bjorn Bakker, Klaske M Schukken, Judith E Simon, Floris Foijer, European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, NL-9713 AV Groningen, The Netherlands

Author contributions: Garcia-Martinez J and Bakker B contributed equally; all authors contributed to this paper.

Supported by The Pediatric Oncology Foundation Groningen (SKOG; http://www.kinderoncologiegroningen.nl); European Union (Marie Curie Innovative Training Network PloidyNet, http://aneuploidy.nl); and Dutch Cancer Society (http://www.kwf.nl grant# 2012-RUG-5549) for funding.

Conflict-of-interest statement: Authors declare that they have no conflict of interest.

Correspondence to: Floris Foijer, PhD, European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, A. Deusinglaan 1, NL-9713 AV Groningen, The Netherlands. f.foijer@umcg.nl
Telephone: +31-50-3617300
Fax: +31-50-3617310

Received: November 17, 2015
Peer-review started: November 23, 2015
First decision: January 18, 2016
Revised: February 18, 2016
Accepted: March 17, 2016
Article in press: March 18, 2016
Published online: June 26, 2016

Abstract

Stem cells hold enormous promise for regenerative medicine as well as for engineering of model systems to study diseases and develop new drugs. The discovery of protocols that allow for generating induced pluripotent stem cells (IPSCs) from somatic cells has brought this promise steps closer to reality. However, as somatic cells might have accumulated various chromosomal abnormalities, including aneuploidies throughout their lives, the resulting IPSCs might no longer carry the perfect blueprint for the tissue to be generated, or worse, become at risk of adopting a malignant fate. In this review, we discuss the contribution of aneuploidy to healthy tissues and how aneuploidy can lead to disease. Furthermore, we review the differences between how somatic cells and stem cells respond to aneuploidy.

Key words: Chromosomal instability; Aneuploidy; Embryonic stem cells; Induced pluripotent stem cells; Mesenchymal stem cells

Core tip: Stem cells hold great therapeutic promise for regenerative medicine, especially with new protocols that can create induced pluripotent stem cells from terminally differentiated cells. However, somatic cells and stem cells cope differently with genomic instability. Therefore, it will be of the utmost importance to assess genomic integrity when preparing stem cell cultures for future therapy.

Garcia-Martinez J, Bakker B, Schukken KM, Simon JE, Foijer F. Aneuploidy in stem cells. *World J Stem Cells* 2016; 8(6): 216-222 Available from: URL: http://www.wjgnet.com/1948-0210/full/v8/i6/216.htm DOI: http://dx.doi.org/10.4252/wjsc.v8.i6.216

INTRODUCTION

Chromosomal instability (CIN) is a process that leads to cells with unbalanced genomes, containing structural

abnormalities (*i.e.,* structural CIN leading to amplification, deletions, translocations), numerical abnormalities (*i.e.,* numerical CIN), or both. Numerical CIN leads to aneuploidy, a state in which cells have abnormal numbers of whole chromosomes. While the majority of all cancers are aneuploid[1], aneuploidy itself appears to act anti-proliferative in non-transformed cells, suggesting that cancer cells somehow have adjusted to the adverse effects of aneuploidy[2,3]. Emerging evidence is indicating that aneuploidy in somatic cells increases with age and might even contribute to natural ageing[4-8]. In fact, we are only beginning to understand how our somatic cells and stem cells cope with loss of genomic integrity. As future regenerative medicine-based therapies will likely make use of induced pluripotent stem cells (iPSCs) derived from somatic (aged) patient's cells, aneuploidy will become an important parameter to test for when assessing the quality of the transplanted cells. Furthermore, much is still unknown regarding the effects aneuploidy on the functionality of both somatic stem cells and iPSCs. In this review, we will discuss the impact of aneuploidy on healthy stem cells, how aneuploidy can lead to disease, and how stem cells cope with genomic instability.

ANEUPLOIDY IN HEALTHY TISSUES

Even though systemic aneuploidies or high CIN rates are poorly tolerated during development, various lines of evidence indicate that some of our tissues can cope remarkably well with aneuploidy[9] and even show aneuploidy under "normal" conditions[4]. Two tissues that have been associated with increased aneuploidy frequencies are brain and liver.

Aneuploidy in the brain

Aneuploidy in mature brain can affect both the neuronal and non-neuronal populations of the cortex[10,11], and is thought to originate in ventricular zone progenitors that encounter a variety of cell division defects during embryogenesis[12,13]. While the most common aneuploid event in progenitors is the loss of a single chromosome, progenitors with up to 5 chromosome losses have been reported. Based on this and other work, cumulatively, 10% of neurons in a healthy brain are estimated to be aneuploid[14], but the role and fate of these cells remains unclear. For more extensive review see Bushman *et al*[15].

The precise contribution of aneuploidy to the healthy human brain remains unclear. One possible explanation is that aneuploidy actually serves a function and is part of a "normal" process during brain development resulting in heterogeneous neuronal and glial populations that contribute to the wide functions that neurons can have[16]. An alternative explanation however is that aneuploidy rates have been overestimated due to technical limitations of the protocols used to quantify aneuploidy in neurons (in most cases interphase FISH)[17], which was reinforced by recent studies that used single cell next generation sequencing to determine chromosome copy numbers in adult brains[14,18-20]. Aneuploidy in the brain has furthermore been linked to various pathologies such as Alzheimer's disease (AD) (increased trisomy of chromosome 21), schizophrenia, and autism[21,22]. Further quantification of aneuploidy in the (diseased) human brain is needed to resolve these conflicting observations.

Aneuploidy in the liver

Roughly half of the hepatocytes in an adult liver are polyploid or aneuploid[23]. Interestingly, liver regeneration has been attributed to proliferation of mature hepatocytes, but not liver stem cells[24]. This is in line with other observations that stem cells tolerate aneuploidy poorly[4,9]. The aneuploidization and polyploidization of hepatocytes has been suggested to contribute to a great variety of hepatocyte genotypes that could help the liver adapt to different insults and chronic stressors[23]. While single cell next generation sequencing failed to detect aneuploidy in adult neurons, it did confirm the polyploidy and aneuploidy rates previously measured in hepatocytes[18], further emphasizing the importance of increased efforts to quantify aneuploidy in various tissues using several techniques.

ANEUPLOIDY IN HUMAN PATHOLOGIES

Stem cells and ageing

Functional exhaustion of adult stem cells is an important contributing factor to natural ageing, as stem cells are essential for tissue maintenance, especially in tissues that have a high turnover rate, such as the intestinal wall and the skin[25]. Indeed, ageing coincides with a functional decline of stem cell function in various organs, even though this is not always accompanied by reduced stem cell numbers. For example, in humans and some mouse strains the number of hematopoietic stem cells even increases during ageing[26-28], but their potential to differentiate decreases[28]. However, whether increased aneuploidy rates as observed in ageing tissues[8,29] contribute to stem cells exhaustion needs further testing. There are several syndromes that are caused by systemic aneuploidy that exhibit also premature ageing features, such as Down's syndrome (DS) or Edward's syndrome. However, how the systemic aneuploidy in these patients impacts stem cell integrity and stem cell numbers requires further investigation.

Stable systemic aneuploidy: DS

The most well known condition linked to systemic aneuploidy is DS. This syndrome, caused by a systemic gain of chromosome 21 (trisomy 21), is associated with developmental and cognitive defects and affects about 1 in 700 individuals[30]. In line with a role of aneuploidy in natural ageing[8], DS is associated with an earlier onset of aging-related pathologies such as AD, and an increased incidence of cancer[31]. One proposed driver of the accelerated ageing phenotype in DS is the observed overexpression of *CDKN2A,* as a result of

epigenetic remodulation in various cell lineages including hematopoietic stem cells, mammary epithelium, fibroblasts and neural progenitors cells[32]. Enhanced *CDKN2A* expression is associated with senescence and stem cell self-renewal defects, further emphasizing the link between aneuploidy and ageing.

STEM CELLS AND CIN

Stem cell biology is a rapidly developing field recently revolutionized through the discovery for protocols to revert terminally differentiated cells back into a pluripotent state (IPSCs)[33]. Stem cells hold great therapeutic promise for the treatment of a large number of diseases and are defined as cell lineages that have 3 cardinal features: (1) self-renewal through asymmetric cell division yielding one stem cell and one differentiated cell or symmetric division yielding two stem cells; (2) the capacity to produce multiple cell lineages; and (3) the potential to proliferate extensively[34,35]. We are only beginning to understand how to isolate and maintain stem cells in tissue culture and how to differentiate them into specific tissues, all of which are crucially important to exploit stem cells in future therapies[36].

Stem cell potency

Stem cells can be subdivided in two subtypes that differ in their differentiation potential or potency: (1) Pluripotent stem cells: Embryonic stem cells (ESCs) are pluripotent, which means that they can form any embryonic tissue, embryonic therefore can form a complete and viable organism when injected into a blastocyst. Mouse ESC culture has revolutionized biology as it allowed for making transgenic and knockout mice[37,38]; Pluripotent stem cells can form any of the three germ layers of an embryo. The recent discovery of protocols to induce pluripotency in differentiated cells, yielding IPSCs[33] has propelled the stem cell field as a whole and made this stem cell subtype the current central tool in stem cell research, as, in theory, iPSC protocols would allow us to make any cell type from any patient's somatic cell, overcoming graft versus host disease and omitting the ethical concerns of human ESCs; and (2) The second subtype of stem cells are multipotent, somatic or adult stem cells. These stem cells that can form a number of lineages, typically within one tissue type. Examples of somatic stem cells are neural stem cells, mammary stem cells or hematopoietic stem cells. In many cases somatic stem cells produce unipotent proliferative cells or transit amplifying cells, *e.g.,* through asymmetric stem cell division These cells can still replicate, but only form one cell lineage before cells terminally differentiate and exit the cell cycle[39] and are therefore not considered to be genuine stem cells.

HOW CAN STEM CELLS RESPOND TO ANEUPLOIDY?

Because of their important role in development and tissue maintenance, stem cells have to safeguard their genome integrity. Any genetic alteration that may occur in a dividing stem cell will be inherited by the entire lineage emerging from this single stem cell and thus give rise to severe developmental defects or pathologies. Genomic integrity maintenance in stem cells is not only important for proper embryonic development and adult tissue homeostasis, but also an essential requirement for the use of stem cells in regenerative medicine and research. This is particularly true for mesenchymal stem cells (MSCs), that are already used in therapy. Importantly, both embryonic and somatic stem cells have developed ways to prevent the negative effects of mutations and aneuploidies resulting in an increased DNA damage response in stem cells as compared to somatic cells, thus preventing structural CIN[40].

DNA damage repair systems in stem cells

One way stem cells accomplish an increased DNA damage response is by increasing the expression of genes involved in DNA repair resulting in increased efficiency in repairing DNA lesions, when compared to differentiated cells[40]. The most dangerous form of DNA damage is the formation of DNA double strand breaks (DSBs). These can arise from replication stress, reactive oxygen species (ROS), mutagens and other DNA damaging events. To repair DSBs, cells employ two main DNA repair systems: Non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is an error-prone form of repair taking place in the G1 phase of the cell cycle in which the sequence flanking the break point is resected followed by blunt end ligation of the DNA ends, which by definition results in small deletions in the DNA sequence. In contrast, HR utilizes the sister chromatid as a repair template to fully repair the DSB, and can therefore only occur when sister chromatids are available, in the G2 phase of the cell cycle. Another way by which stem cells maintain genomic fidelity is by their predominant use of HR to repair DSBs, while differentiated (non-proliferating) cells can only use NHEJ, as the latter have exited the cell cycle and therefore have no "access" to duplicated sister chromatids[41,42]. Therefore, the preferred use of HR to repair DNA DSBs combined with more sensitive DNA damage signaling helps stem cells to maintain genomic integrity.

The safe way out: Apoptosis and differentiation

An alternative method to prevent daughter cells from inheriting genomic aberrations is by eliminating the aberrant stem cells from the stem cell pool. This can be done through apoptosis, removing the cell altogether, or by differentiating the compromised stem cell in order to avoid further cell divisions. Indeed, when encountering DNA damage, stem cells activate the p53 pathway to mobilize the DNA repair machinery. When damage remains unrepaired, p53 activity promotes apoptosis. While apoptosis is the main response to DNA damage in stem cells, induction of p53 in human stem cells can

Table 1 Reported chromosomal aberrations in different stem cell types

Species	Stem cell type	Chromosomal aberration
Human	Cultured embryonic stem cells	+1, +12, +14, +17, +20
	Mesenchymal stem cells	4N, -13
		+4, +5, +10, +X
Mouse	Cultured embryonic stem cells	+8

also result in spontaneous differentiation[43], however, which factors determine the switch between these two choices are so far unclear.

This is exemplified further by the ways by which different stem cell types respond to DNA damage. For example, hematopoietic stem cells (HSCs) enhance DNA repair and stem cell maintenance, and prevent apoptosis to avoid depletion of the HSC pool. Intestinal stem cells on the other hand favor increased cell death and melanocytes respond by terminal differentiation[44]. Therefore, different responses to DNA damage in different stem cell populations will yield different effects on the integrity of the stem cell pool and their downstream potencies.

STEM CELLS COPE POORLY WITH NUMERICAL CIN

Numerical CIN and the resulting aneuploidy is detrimental for embryonic development, evidenced by early embryonic death observed in mouse models in which high grade numerical CIN was provoked systemically, see for extensive review[45-47]. While these observations led to the prevailing view that high grade numerical CIN is never tolerated, more recent observations are nuancing this view. For instance, when numerical CIN was provoked in a tissue specific fashion, in mouse epidermis, epidermal hair follicle stem cells were rapidly depleted, while the more committed transit amplifying (unipotent) cells tolerated the resulting high grade aneuploidy remarkably well[9]. Furthermore, while aneuploid cells seem to accumulate in various somatic cell types in the ageing mouse, aneuploidy in stem cell linages in the same mice remains rare, further indicating that stem cells are well protected against (or ultra sensitive to) numerical chromosome abnormalities[4]. However, even though these observations are suggestive of an aneuploidy checkpoint in stem cells, more work is needed to reveal if this checkpoint does exist and if so, how this checkpoint operates.

DO CULTURE CONDITIONS IMPACT STEM CELL GENOMIC INTEGRITY?

As stem cells are typically isolated in small quantities and iPSC protocols are still quite inefficient, stem cells are exposed to a period of tissue culture stress that can yield (further) CIN. While the majority of such alterations will be negatively selected and therefore disappear within a few passages, some genetic alterations could result in proliferative advantages and outcompete the "normal" stem cells, a process known as "culture adaptation"[48] resulting in late passage cultures that grow better and show better plating efficiencies[49].

Frequently reported chromosome aberrations in stem cells

Various cytogenetic abnormalities have been reported in cultured ESCs, with varying frequencies in different cell strains. The most common abnormality reported in murine ESCs is trisomy of chromosome 8 (Table 1). Trisomies of chromosome 12, 14 and 17 are most frequently observed in human ESCs[50], but not necessarily within the same ES cell line. Further studies that investigated the genomic integrity of > 150 human ES cell lines and 220 iPSC lines found that, while the majority of the stem cell lines were euploid, approximately 12%-13% of human ES cell lines and similar fractions of iPSC lines showed whole chromosome abnormalities[49,51].

Notably in ESC culture, chromosomal abnormalities increased with prolonged culturing as late passage cultures had approximately twice as many aberrations, which typically involved gain of chromosomes[49]. In line with culture adaptation, chromosome aberrations were non-random with 60% of the aneuploid stem cell cultures showing abnormalities for chromosomes 1, 12, 17 and/or 20 (Table 1)[49]. Chromosome 12 gain has been observed by others as well[52,53] and although selection for chromosome 12 is likely to be driven by multiple genes, it is tempting to speculate that *NANOG* is a key driver for this commonly observed trisomy in stem cell cultures, possibly together with the pluripotency related *DPPA3* and *GDF3*, and the cell cycle regulator *CCND2*. Furthermore, the oncogene *KRAS* is also located on chromosome 12[49,54]. Importantly, culture conditions appear to also have an important impact on genomic integrity of stem cell cultures, for instance the use of fetal bovine serum as growth supplement results in increased chromosomal abnormalities[55]. Last, but certainly not least important, iPS cells are typically derived from somatic (differentiated cells) that appear to tolerate aneuploidy much better than previously anticipated[9] and therefore the founder cells could already have been aneuploid to start out with. Aneuploidies in these founder cells might lower IPSC protocol's efficiency if the induced stem cells do not tolerate the aneuploidies. Even worse, when aneuploidies in the founder cells are subtle, the aneuploidies could be maintained in the resulting IPSC clones.

As MSCs are frequently used in therapy, their genomic stability is routinely assessed. The most commonly reported type of aberration in MSCs several passages after cell expansion is tetraploidization, observed in 6 out 21 patient-derived cell lines[56]. Tetraploidy was observed only in a minority of examined metaphases (approximately 1 in 20-30 per cell line), and whether the contribution of these tetraploid cells will have significant consequences *in vivo* needs further investigation. Other reported aberrations include a preferential loss of chromosome 13 in later passages of the human MSC line UE6E7T-3 (Table 1)[57],

and the emergence of clonal trisomies for chromosomes 4, 5, 10, and X in a male patient-derived MSC[58]. Therefore, while cytogenetic aberrations are widely reported for MSCs, their ultimate effect on MSC proliferation and potency *in vivo* are less well understood. This also holds true for other stem cell populations *in vivo.* Novel karyotyping methods (for extensive review see Bakker *et al*[17]) that circumvent the limitations of existing techniques will be instrumental in resolving both the incidence and effects of aneuploidy in adult stem cells in various different tissues.

CONCLUSION

Stem cells are the origin of tissue homeostasis and therefore crucially rely on genomic integrity. Fortunately, stem cells appear to be much more sensitive to DNA damage and aneuploidy than their differentiated counterparts[9,40-42]. However, as IPSC protocols force terminally differentiated cells back into the cell cycle, cells might accumulate mutations in the process of becoming pluripotent, or even harbor chromosomal abnormalities before dedifferentiation starts. Therefore, as IPSC protocols are becoming the new standard in regenerative medicine, it will be of the utmost importance to develop sequencing pipelines that can ensure chromosomal fidelity of the engineered IPSCs or IPSC-derived tissues.

REFERENCES

1 **Duijf PH**, Schultz N, Benezra R. Cancer cells preferentially lose small chromosomes. *Int J Cancer* 2013; **132**: 2316-2326 [PMID: 23124507 DOI: 10.1002/ijc.27924]

2 **Williams BR**, Prabhu VR, Hunter KE, Glazier CM, Whittaker CA, Housman DE, Amon A. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science* 2008; **322**: 703-709 [PMID: 18974345 DOI: 10.1126/science.1160058]

3 **Torres EM**, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, Amon A. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* 2007; **317**: 916-924 [PMID: 17702937 DOI: 10.1126/science.1142210]

4 **Baker DJ**, Dawlaty MM, Wijshake T, Jeganathan KB, Malureanu L, van Ree JH, Crespo-Diaz R, Reyes S, Seaburg L, Shapiro V, Behfar A, Terzic A, van de Sluis B, van Deursen JM. Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. *Nat Cell Biol* 2013; **15**: 96-102 [PMID: 23242215 DOI: 10.1038/ncb2643]

5 **Baker DJ**, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 2011; **479**: 232-236 [PMID: 22048312 DOI: 10.1038/nature10600]

6 **Baker DJ**, Jeganathan KB, Malureanu L, Perez-Terzic C, Terzic A, van Deursen JM. Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. *J Cell Biol* 2006; **172**: 529-540 [PMID: 16476774 DOI: 10.1083/jcb.200507081]

7 **Baker DJ**, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P, van Deursen JM. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet* 2004; **36**: 744-749 [PMID: 15208629 DOI: 10.1038/ng1382]

8 **Thomas P**, Fenech M. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. *Mutagenesis* 2008; **23**: 57-65 [PMID: 18048581 DOI: 10.1093/mutage/gem044]

9 **Foijer F**, DiTommaso T, Donati G, Hautaviita K, Xie SZ, Heath E, Smyth I, Watt FM, Sorger PK, Bradley A. Spindle checkpoint deficiency is tolerated by murine epidermal cells but not hair follicle stem cells. *Proc Natl Acad Sci USA* 2013; **110**: 2928-2933 [PMID: 23382243 DOI: 10.1073/pnas.1217388110]

10 **Rehen SK**, Yung YC, McCreight MP, Kaushal D, Yang AH, Almeida BS, Kingsbury MA, Cabral KM, McConnell MJ, Anliker B, Fontanoz M, Chun J. Constitutional aneuploidy in the normal human brain. *J Neurosci* 2005; **25**: 2176-2180 [PMID: 15745943 DOI: 10.1523/JNEUROSCI.4560-04.2005]

11 **Yurov YB**, Iourov IY, Monakhov VV, Soloviev IV, Vostrikov VM, Vorsanova SG. The variation of aneuploidy frequency in the developing and adult human brain revealed by an interphase FISH study. *J Histochem Cytochem* 2005; **53**: 385-390 [PMID: 15750026 DOI: 10.1369/jhc.4A6430.2005]

12 **Rehen SK**, McConnell MJ, Kaushal D, Kingsbury MA, Yang AH, Chun J. Chromosomal variation in neurons of the developing and adult mammalian nervous system. *Proc Natl Acad Sci USA* 2001; **98**: 13361-13366 [PMID: 11698687 DOI: 10.1073/pnas.231487398]

13 **Yang AH**, Kaushal D, Rehen SK, Kriedt K, Kingsbury MA, McConnell MJ, Chun J. Chromosome segregation defects contribute to aneuploidy in normal neural progenitor cells. *J Neurosci* 2003; **23**: 10454-10462 [PMID: 14614104]

14 **McConnell MJ**, Lindberg MR, Brennand KJ, Piper JC, Voet T, Cowing-Zitron C, Shumilina S, Lasken RS, Vermeesch JR, Hall IM, Gage FH. Mosaic copy number variation in human neurons. *Science* 2013; **342**: 632-637 [PMID: 24179226 DOI: 10.1126/science.1243472]

15 **Bushman DM**, Chun J. The genomically mosaic brain: aneuploidy and more in neural diversity and disease. *Semin Cell Dev Biol* 2013; **24**: 357-369 [PMID: 23466288 DOI: 10.1016/j.semcdb.2013.02.003]

16 **Westra JW**, Peterson SE, Yung YC, Mutoh T, Barral S, Chun J. Aneuploid mosaicism in the developing and adult cerebellar cortex. *J Comp Neurol* 2008; **507**: 1944-1951 [PMID: 18273885 DOI: 10.1002/cne.21648]

17 **Bakker B**, van den Bos H, Lansdorp PM, Foijer F. How to count chromosomes in a cell: An overview of current and novel technologies. *Bioessays* 2015; **37**: 570-577 [PMID: 25739518 DOI: 10.1002/bies.201400218]

18 **Knouse KA**, Wu J, Whittaker CA, Amon A. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. *Proc Natl Acad Sci USA* 2014; **111**: 13409-13414 [PMID: 25197050 DOI: 10.1073/pnas.1415287111]

19 **Knouse KA**, Wu J, Amon A. Assessment of megabase-scale somatic copy number variation using single-cell sequencing. *Genome Res* 2016; **26**: 376-384 [PMID: 26772196 DOI: 10.1101/gr.198937.115]

20 **Cai X**, Evrony GD, Lehmann HS, Elhosary PC, Mehta BK, Poduri A, Walsh CA. Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain. *Cell Rep* 2015; **10**: 645 [PMID: 25832109 DOI: 10.1016/j.celrep.2014.07.043]

21 **Vorsanova SG**, Yurov IY, Demidova IA, Voinova-Ulas VY, Kravets VS, Solov'ev IV, Gorbachevskaya NL, Yurov YB. Variability in the heterochromatin regions of the chromosomes and chromosomal anomalies in children with autism: identification of genetic markers of autistic spectrum disorders. *Neurosci Behav Physiol* 2007; **37**: 553-558 [PMID: 17657425 DOI: 10.1007/s11055-007-0052-1]

22 **Yurov YB**, Iourov IY, Vorsanova SG, Demidova IA, Kravetz VS, Beresheva AK, Kolotii AD, Monakchov VV, Uranova NA, Vostrikov VM, Soloviev IV, Liehr T. The schizophrenia brain exhibits low-level aneuploidy involving chromosome 1. *Schizophr Res* 2008; **98**: 139-147 [PMID: 17889509 DOI: 10.1016/j.schres.2007.07.035]

23 **Duncan AW**, Hanlon Newell AE, Smith L, Wilson EM, Olson SB, Thayer MJ, Strom SC, Grompe M. Frequent aneuploidy among normal human hepatocytes. *Gastroenterology* 2012; **142**: 25-28

[PMID: 22057114 DOI: 10.1053/j.gastro.2011.10.029]
24 **Miyaoka Y**, Ebato K, Kato H, Arakawa S, Shimizu S, Miyajima A. Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. *Curr Biol* 2012; **22**: 1166-1175 [PMID: 22658593 DOI: 10.1016/j.cub.2012.05.016]
25 **Rando TA**. Stem cells, ageing and the quest for immortality. *Nature* 2006; **441**: 1080-1086 [PMID: 16810243 DOI: 10.1038/nature04958]
26 **Pang WW**, Price EA, Sahoo D, Beerman I, Maloney WJ, Rossi DJ, Schrier SL, Weissman IL. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc Natl Acad Sci USA* 2011; **108**: 20012-20017 [PMID: 22123971 DOI: 10.1073/pnas.1116110108]
27 **Rossi DJ**, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, Weissman IL. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci USA* 2005; **102**: 9194-9199 [PMID: 15967997 DOI: 10.1073/pnas.0503280102]
28 **Sudo K**, Ema H, Morita Y, Nakauchi H. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med* 2000; **192**: 1273-1280 [PMID: 11067876 DOI: 10.1084/jem.192.9.1273]
29 **Simon JE**, Bakker B, Foijer F. CINcere Modelling: What Have Mouse Models for Chromosome Instability Taught Us? *Recent Results Cancer Res* 2015; **200**: 39-60 [PMID: 26376871 DOI: 10.1007/978-3-319-20291-4_2]
30 **Antonarakis SE**, Lyle R, Dermitzakis ET, Reymond A, Deutsch S. Chromosome 21 and down syndrome: from genomics to pathophysiology. *Nat Rev Genet* 2004; **5**: 725-738 [PMID: 15510164 DOI: 10.1038/nrg1448]
31 **Roth GM**, Sun B, Greensite FS, Lott IT, Dietrich RB. Premature aging in persons with Down syndrome: MR findings. *AJNR Am J Neuroradiol* 1996; **17**: 1283-1289 [PMID: 8871713]
32 **Adorno M**, Sikandar S, Mitra SS, Kuo A, Nicolis Di Robilant B, Haro-Acosta V, Ouadah Y, Quarta M, Rodriguez J, Qian D, Reddy VM, Cheshier S, Garner CC, Clarke MF. Usp16 contributes to somatic stem-cell defects in Down's syndrome. *Nature* 2013; **501**: 380-384 [PMID: 24025767 DOI: 10.1038/nature12530]
33 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]
34 **Al-Hajj M**, Clarke MF. Self-renewal and solid tumor stem cells. *Oncogene* 2004; **23**: 7274-7282 [PMID: 15378087 DOI: 10.1038/sj.onc.1207947]
35 **Pardal R**, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer* 2003; **3**: 895-902 [PMID: 14737120 DOI: 10.1038/nrc1232]
36 **Ben-David U**, Kopper O, Benvenisty N. Expanding the boundaries of embryonic stem cells. *Cell Stem Cell* 2012; **10**: 666-677 [PMID: 22704506 DOI: 10.1016/j.stem.2012.05.003]
37 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]
38 **van der Weyden L**, Shaw-Smith C, Bradley A. Chromosome engineering in ES cells. *Methods Mol Biol* 2009; **530**: 49-77 [PMID: 19266329 DOI: 10.1007/978-1-59745-471-1_4]
39 **Slack JM**. Stem cells in epithelial tissues. *Science* 2000; **287**: 1431-1433 [PMID: 10688782 DOI: 10.1126/science.287.5457.1431]
40 **Maynard S**, Swistowska AM, Lee JW, Liu Y, Liu ST, Da Cruz AB, Rao M, de Souza-Pinto NC, Zeng X, Bohr VA. Human embryonic stem cells have enhanced repair of multiple forms of DNA damage. *Stem Cells* 2008; **26**: 2266-2274 [PMID: 18566332 DOI: 10.1634/stemcells.2007-1041]
41 **Tichy ED**, Pillai R, Deng L, Liang L, Tischfield J, Schwemberger SJ, Babcock GF, Stambrook PJ. Mouse embryonic stem cells, but not somatic cells, predominantly use homologous recombination to repair double-strand DNA breaks. *Stem Cells Dev* 2010; **19**: 1699-1711 [PMID: 20446816 DOI: 10.1089/scd.2010.0058]
42 **Baker DJ**, Jin F, Jeganathan KB, van Deursen JM. Whole chromosome instability caused by Bub1 insufficiency drives tumorigenesis through tumor suppressor gene loss of heterozygosity. *Cancer Cell* 2009; **16**: 475-486 [PMID: 19962666 DOI: 10.1016/j.ccr.2009.10.023]
43 **Jain AK**, Allton K, Iacovino M, Mahen E, Milczarek RJ, Zwaka TP, Kyba M, Barton MC. p53 regulates cell cycle and microRNAs to promote differentiation of human embryonic stem cells. *PLoS Biol* 2012; **10**: e1001268 [PMID: 22389628 DOI: 10.1371/journal.pbio.1001268]
44 **Alizadeh AA**, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, Caldas C, Califano A, Doherty M, Elsner M, Esteller M, Fitzgerald R, Korbel JO, Lichter P, Mason CE, Navin N, Pe'er D, Polyak K, Roberts CW, Siu L, Snyder A, Stower H, Swanton C, Verhaak RG, Zenklusen JC, Zuber J, Zucman-Rossi J. Toward understanding and exploiting tumor heterogeneity. *Nat Med* 2015; **21**: 846-853 [PMID: 26248267 DOI: 10.1038/nm.3915]
45 **Holland AJ**, Cleveland DW. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat Rev Mol Cell Biol* 2009; **10**: 478-487 [PMID: 19546858 DOI: 10.1038/nrm2718]
46 **Schvartzman JM**, Sotillo R, Benezra R. Mitotic chromosomal instability and cancer: mouse modelling of the human disease. *Nat Rev Cancer* 2010; **10**: 102-115 [PMID: 20094045 DOI: 10.1038/nrc2781]
47 **Foijer F**, Draviam VM, Sorger PK. Studying chromosome instability in the mouse. *Biochim Biophys Acta* 2008; **1786**: 73-82 [PMID: 18706976 DOI: 10.1016/j.bbcan.2008.07.004]
48 **Enver T**, Soneji S, Joshi C, Brown J, Iborra F, Orntoft T, Thykjaer T, Maltby E, Smith K, Abu Dawud R, Jones M, Matin M, Gokhale P, Draper J, Andrews PW. Cellular differentiation hierarchies in normal and culture-adapted human embryonic stem cells. *Hum Mol Genet* 2005; **14**: 3129-3140 [PMID: 16159889 DOI: 10.1093/hmg/ddi345]
49 **Amps K**, Andrews PW, Anyfantis G, Armstrong L, Avery S, Baharvand H, Baker J, Baker D, Munoz MB, Beil S, Benvenisty N, Ben-Yosef D, Biancotti JC, Bosman A, Brena RM, Brison D, Caisander G, Camarasa MV, Chen J, Chiao E, Choi YM, Choo AB, Collins D, Colman A, Crook JM, Daley GQ, Dalton A, De Sousa PA, Denning C, Downie J, Dvorak P, Montgomery KD, Feki A, Ford A, Fox V, Fraga AM, Frumkin T, Ge L, Gokhale PJ, Golan-Lev T, Gourabi H, Gropp M, Lu G, Hampl A, Harron K, Healy L, Herath W, Holm F, Hovatta O, Hyllner J, Inamdar MS, Irwanto AK, Ishii T, Jaconi M, Jin Y, Kimber S, Kiselev S, Knowles BB, Kopper O, Kukharenko V, Kuliev A, Lagarkova MA, Laird PW, Lako M, Laslett AL, Lavon N, Lee DR, Lee JE, Li C, Lim LS, Ludwig TE, Ma Y, Maltby E, Mateizel I, Mayshar Y, Mileikovsky M, Minger SL, Miyazaki T, Moon SY, Moore H, Mummery C, Nagy A, Nakatsuji N, Narwani K, Oh SK, Oh SK, Olson C, Otonkoski T, Pan F, Park IH, Pells S, Pera MF, Pereira LV, Qi O, Raj GS, Reubinoff B, Robins A, Robson P, Rossant J, Salekdeh GH, Schulz TC, Sermon K, Sheik Mohamed J, Shen H, Sherrer E, Sidhu K, Sivarajah S, Skottman H, Spits C, Stacey GN, Strehl R, Strelchenko N, Suemori H, Sun B, Suuronen R, Takahashi K, Tuuri T, Venu P, Verlinsky Y, Ward-van Oostwaard D, Weisenberger DJ, Wu Y, Yamanaka S, Young L, Zhou Q. Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. *Nat Biotechnol* 2011; **29**: 1132-1144 [PMID: 22119741 DOI: 10.1038/nbt.2051]
50 **Rajamani K**, Li YS, Hsieh DK, Lin SZ, Harn HJ, Chiou TW. Genetic and epigenetic instability of stem cells. *Cell Transplant* 2014; **23**: 417-433 [PMID: 24622296 DOI: 10.3727/096368914X678472]
51 **Taapken SM**, Nisler BS, Newton MA, Sampsell-Barron TL, Leonhard KA, McIntire EM, Montgomery KD. Karotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. *Nat Biotechnol* 2011; **29**: 313-314 [PMID: 21478842 DOI: 10.1038/nbt.1835]
52 **Cowan CA**, Klimanskaya I, McMahon J, Atienza J, Witmyer J, Zucker JP, Wang S, Morton CC, McMahon AP, Powers D, Melton DA. Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med* 2004; **350**: 1353-1356 [PMID: 14999088 DOI: 10.1056/NEJMsr040330]
53 **Draper JS**, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J,

Meisner L, Zwaka TP, Thomson JA, Andrews PW. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol* 2004; **22**: 53-54 [PMID: 14661028 DOI: 10.1038/nbt922]

54 **Na J**, Baker D, Zhang J, Andrews PW, Barbaric I. Aneuploidy in pluripotent stem cells and implications for cancerous transformation. *Protein Cell* 2014; **5**: 569-579 [PMID: 24899134 DOI: 10.1007/s13238-014-0073-9]

55 **Dahl JA**, Duggal S, Coulston N, Millar D, Melki J, Shahdadfar A, Brinchmann JE, Collas P. Genetic and epigenetic instability of human bone marrow mesenchymal stem cells expanded in autologous serum or fetal bovine serum. *Int J Dev Biol* 2008; **52**: 1033-1042 [PMID: 18956336 DOI: 10.1387/ijdb.082663jd]

56 **Borgonovo T**, Vaz IM, Senegaglia AC, Rebelatto CL, Brofman PR. Genetic evaluation of mesenchymal stem cells by G-banded karyotyping in a Cell Technology Center. *Rev Bras Hematol Hemoter* 2014; **36**: 202-207 [PMID: 25031060 DOI: 10.1016/j.bjhh.2014.03.006]

57 **Takeuchi M**, Takeuchi K, Ozawa Y, Kohara A, Mizusawa H. Aneuploidy in immortalized human mesenchymal stem cells with non-random loss of chromosome 13 in culture. *In Vitro Cell Dev Biol Anim* 2009; **45**: 290-299 [PMID: 19184247 DOI: 10.1007/s11626-008-9174-1]

58 **Borgonovo T**, Solarewicz MM, Vaz IM, Daga D, Rebelatto CL, Senegaglia AC, Ribeiro E, Cavalli IJ, Brofman PS. Emergence of clonal chromosomal alterations during the mesenchymal stromal cell cultivation. *Mol Cytogenet* 2015; **8**: 94 [PMID: 26628918 DOI: 10.1186/s13039-015-0197-5]

P- Reviewer: Garcia-Olmo D, Gunther T, Mustapha N
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com