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ACQUIRED GENETIC CHANGES IN PLURIPOTENT STEM CELLS: ORIGINS AND CONSEQUENCES

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ABSTRACT (200 words)

In the twenty years since human embryonic stem cells, and subsequently induced pluripotent stem cells (collectively, pluripotent stem cells), were first described, it has become apparent that these cells may acquire genetic changes during long term culture, commonly manifest by gains or losses of particular chromosomal regions, or by mutations in certain cancer associated genes, especially *TP53*. Such changes raise concerns for the safety of products destined for clinical applications in regenerative medicine. Although acquired changes may not be present in a cell line at the start of a research program, the low sensitivity of current detection methods means that mutations may be difficult to detect if they arise but are only present in a small proportion of the cells. Nevertheless, recent work suggests that the underlying mutation rate in pluripotent stem cells is low, though they also seem to be particularly susceptible to genomic damage. This apparent contradiction can be reconciled by the observations that, in contrast to somatic cells, pluripotent stem cells are programmed to die in response to genomic damage, which may reflect the requirements of early embryogenesis. Thus, the common variants that do occur are likely rare events that offer the cells a selective growth advantage.

INTRODUCTION

Although little more than 20 years has passed since the first human embryonic stem cells (ESC) were reported (Thomson et al., 1998, Reubinoff et al., 2000), and less than 14 years since human induced pluripotent cells (iPSC) were described (Takahashi et al., 2007, Yu et al., 2007), clinical trials for regenerative medicine using derivatives of these cells are already underway or on the horizon (da Cruz et al., 2018, Schwartz et al., 2012, Song et al., 2015, Mandai et al., 2017, Barker et al., 2017). Yet over this period it has become evident that both human ESC and iPSC (collectively here denoted as PSC), although mostly diploid when first derived, may acquire genetic alterations, ranging from large scale structural modifications readily recognized as karyotypic variants through to single base pair changes on subsequent passage (**Figure 1)**. Although not the focus of this review, it is worth noting that epigenetic changes encompassing aberrations in DNA methylation, imprinting and X-chromosome inactivation have also been reported in PSC (reviewed in Bar and Benvenisty, 2019). The observation of genetic and epigenetic changes in PSC has triggered worries about the significance of such variants for the safety of PSC-based regenerative medicine (Yasuda et al., 2018, Sato et al., 2019). In particular, some recurrent genetic changes, for example mutations in *TP53*, or gains of chromosome 12p, 17q, and 20q have been associated with various cancers, notably the association of gain of chromosome 12p with embryonal carcinoma (EC) cells, the malignant counterpart of PSC (Andrews, 2002). Indeed, a planned trial of iPSC-derived retinal pigment cells to treat age related macular degeneration was halted when a point mutation was detected, although whether this particular mutation may have caused a problem was unknown (Mandai et al., 2017, Garber, 2015).

Some of the genetic variants found in PSC may well have been present in the embryos or somatic cells from which they were derived, or may have been induced during derivation (Hussein et al., 2011, Rouhani et al., 2016). The outcomes of the currently pursued in-depth studies into the effect of the choice of a starting cell type and a method of reprogramming on the overall mutational burden in iPSC (reviewed in Steichen et al., 2019) will have an important bearing on the practical applications of PSC. However, regardless of their ultimate findings, a key feature of such 'Mutations of Origin' is that they will be present in all the cells of a given PSC line and so should be readily detectable and assessed for their significance before research with a particular line is initiated. Much more problematic, and the subject of this review, are mutations that were not present initially but arise, often recurrently, during culture of the cells – 'Acquired Mutations'. Nevertheless, it is important to view these variants in perspective: many PSC lines do not acquire the commonly observed variants, or only in late passage. In a study by the International Stem Cell Initiative, 79 lines out of 122 retained a normal karyotype (Amps et al., 2011). In another study, recurrent mutants of *TP53* were detected in only five out of 140 human PSC lines (Merkle et al., 2017). On the other hand, since the sensitivity of detecting mutant cells in a mosaic culture is low (see **BOX 1**), such variant cells may lurk in cultures for a considerable time until they take over due to a selective growth advantage, or the line is subject to a population bottleneck. Further, some variants, for example gains of a small region of the long arm of chromosome 20, are particularly difficult to detect by G-banding karyotyping and may go unnoticed, even when present in a substantial proportion of the cells (Amps et al., 2011).

Over the years, many studies have sought to find ways to minimize the appearance of genetic variants in PSC. However, in such quests, it is important to recall that the appearance of the common recurrent variants depends upon two independent events, mutation followed by subsequent selection (**Figure 1**), and these can be experimentally difficult to disentangle. The mechanisms of selective growth advantage, due to the altered expression or activity of one or more genes ('Driver' genes), or to the effects of culture conditions on selection, can be relatively easily analysed by spiking wild type cultures with variant cells and monitoring their subsequent growth patterns (Olariu et al., 2010). By contrast, mutation occurs at a very low frequency so that mutations are difficult to monitor directly without expansion of the variant cells, in which case the estimates of mutation rate may be compromised by selection unless this is avoided by often cumbersome clonogenic strategies (Thompson et al., 2020).

In this review we discuss the nature of the acquired genetic variants that commonly arise during culture of human PSC cultures and consider the potential consequences of acquired genetic variants in human PSC, both for research and for clinical applications. We then discuss the mechanisms of selective growth advantage that lead to the recurrent appearance of particular variants. Finally, we focus on the underlying mechanisms of mutation in PSC; it seems that PSC differ substantially from somatic cells in both their susceptibility and response to DNA damage, which may reflect the exigencies of cell proliferation in the early embryo.

ACQUIRED GENETIC VARIATION

Karyotypic abnormalities

Traditionally, routine screening of PSC lines for the identification of genetic changes has been performed mainly by cytogenetic and molecular methods that are capable of detecting numerical and structural aneuploidies rather than DNA sequence changes (Draper et al., 2004, Amps et al., 2011). Consequently, karyotypic abnormalities are the most comprehensively catalogued genetic changes in PSC to date. In taking stock of the reports of karyotypic abnormalities in PSC over the last two decades, it is clear that the aberrant PSC karyotypes can encompass virtually any type of an abnormality, including numerical aneuploidies, such as a whole chromosome gain (trisomy) or loss (monosomy), as well as structural aneuploidies, including interstitial duplications, deletions, inversions, amplifications and translocations (Draper et al., 2004, Amps et al., 2011, Taapken et al., 2011). That said, the distribution of chromosomal aberrations appears to be non-random and certain types of variants are more commonly seen (Taapken et al., 2011, Draper et al., 2004, Amps et al., 2011, Baker et al., 2016). The first apparent bias is towards gains rather than losses of chromosomal material. Indeed, it is estimated that over 70% of all karyotypic abnormalities reported in ESC represent whole or partial chromosome gains, whereas only around 20% of reported abnormalities are losses of chromosomes or chromosomal material (Baker et al., 2016). Losses of entire chromosomes are particularly rare, representing only around 2% of reported abnormalities in PSC cultures (Baker et al., 2016). The under representation of monosomies in PSC concurs with the observation that cells in general tolerate gains of genetic material more readily than losses (Torres et al., 2008). Both unbalanced and balanced translocations, i.e. with or without the overt net gain or loss of chromosomal material, respectively, have also been reported, but unlike in certain haematological malignancies, for example, no common recurrent translocations or fusion genes have so far been associated with variant PSC (Draper et al., 2004, Amps et al., 2011, Baker et al., 2016, Assou et al., 2020). On the other hand, some chromosomes are rarely, if ever, reported as gained or lost in PSC, including chromosomes 2, 4, 19 and 21 (Amps et al., 2011, Baker et al., 2016). Finally, most striking is the observation of a consistent pattern of chromosomes affected by aneuploidy in PSC, with the majority of detected aberrations in PSC karyotypes representing gains of the whole or fragments of chromosomes 1, 12, 17, 20 and X (Baker et al., 2007, Amps et al., 2011, Nguyen et al., 2013, Baker et al., 2016, Assou et al., 2020) (**Box 2**). Of these, a particularly insidious change is the frequent gain of a small, variable region of located near the centromere of the long arm of chromosome 20 (Lefort et al., 2008, Spits et al., 2008). Often this gain is below the resolution of G-band karyotyping but, for example, it was noted by SNP array analysis in over 20% of the cell lines in the ISCI study (Amps et al., 2011, Baker et al., 2016). That the same repertoire of aneuploidies is observed across different ESC and iPSC lines and across different laboratories world-wide (Amps et al., 2011) points to the enhanced fitness of such variant cells, likely due to an

increased expression of one or more of the genes located on amplified chromosomes (Enver et al., 2005, Ben-David et al., 2014).

Point mutations

Point mutation screening has not yet become routine in PSC maintenance and, therefore, culture acquired nucleotide changes in these cells remain largely unexplored. A couple of, recent studies have investigated the presence and potential recurrence of cancer-related point mutations in PSC. Merkle *et al*. (Merkle et al., 2017) subjected the DNA from 140 ESC lines provided by different laboratories world-wide to whole exome sequencing. After filtering out inherited polymorphisms and focusing only on variants that were present in a subset of cells, as suspected culture-acquired mutations, 28 of the 263 mosaic variants detected across the 140 lines were predicted to alter gene function. Of these, the tumor suppressor gene *TP53* was the only gene in which mutations were detected in multiple cell lines, with six different missense mutations in five independent ESC lines. All of the identified *TP53* missense mutations affected cytosines of highly mutable CpG dinucleotides within four of the residues encoding the DNA-binding domain of the TP53 protein, therefore rendering the mutant TP53 protein inactive. Further *TP53* mutations in both ESC and iPSC were uncovered by leveraging RNA-sequencing datasets from public repositories (Merkle et al., 2017, Avior et al., 2019), thereby establishing *TP53* as a recurrently mutated gene in PSC. As the observed *TP53* mutations represent some of the most frequent mutations in cancer (Kandoth et al., 2013) and are also known to cause a familial cancer predisposition disorder, the Li-Fraumeni syndrome (Malkin et al., 1990, Srivastava et al., 1990), these findings have brought into focus the need for monitoring of PSC for culture-acquired *TP53* mutations.

Despite being the most prevalent, *TP53* mutations are not the only recurrent point mutations arising in cancer-related genes upon PSC expansion (Avior et al., 2019). Recently, recurrent point mutations were detected in at least 22 other genes that were previously classified within the COSMIC Census database as genes with a documented cancer-related activity, including *CCND2*, *PCM1*, *MYH9*, *HIF1A*, *BCL9* and *VHL* (Avior et al., 2019). Intriguingly, the mutational burden seems to differ between different pluripotent states; human PSC in the naïve state, representing the pluripotent state of the pre-implantation epiblast cells (Nichols and Smith, 2009), were estimated to carry four times more mutations than their primed counterparts, which correspond to the pluripotent state of the post-implantation epiblast (Avior et al., 2019). As the naïve samples analysed in this study were reset from primed PSC, rather than derived directly from embryos, the mutational load differences may not be intrinsic to different cell states, but may in fact reflect a substantial selection pressure imposed on the cells during resetting to naïve pluripotency. Supporting the latter view, the genes found to be mutated in the naïve cells were in pathways affected by chemical inhibitors used in resetting primed cells to the naïve state(Avior et al., 2019).

The studies by Merkle et al (2017) and Avior et al (2019) offered an important insight into the mutational landscape of PSC, albeit they focused on analysing a relatively small portion of the PSC genome (i.e. the exome, which represents only about 1% of the genome). Undoubtedly, uncovering the true extent and pattern of point mutations arising in PSC will require much larger, ideally longitudinal datasets, and scrutiny of PSC sequence changes at a genome-wide level. Important for this endeavour will be the implementation of next generation sequencing as a component of routine monitoring of PSC genomes. Currently, the turnaround time and cost of sequencing preclude its use as a routine screening method, but the ongoing technological developments, which are driving down the cost and the data processing time, make this a feasible prospect for the coming decade. Nonetheless, we must remain cognizant of the fact that the reliable detection of mutations is only the first step in handling culture-acquired genetic changes. A far more difficult hurdle is ascribing the functional meaning to the detected mutations, and predicting their potential impact for applications of human PSC.

Consequences for applications of human PSC

The close relationship between experimentally-derived PSC and EC cells, the malignant stem cells of teratocarcinomas, which occur predominantly as testicular germ cell tumors in young men (Andrews, 2002, Mostofi and Price, 1973, Damjanov and Solter, 1974), and the ability of PSC to produce teratomas when grown in immunodeficient mice, has always provoked concerns that cancer presents a significant safety hazard for PSC based regenerative medicine. However, it is important to recognize the distinction between teratomas and teratocarcinomas. Teratomas are tumors containing differentiated cells without any persisting PSC. By contrast, teratocarcinomas are tumors with the characteristics of teratomas that also contain undifferentiated PSC (**Figure 2**) (Damjanov and Andrews, 2007b, Damjanov and Andrews, 2016). Clinically, teratocarcinomas are highly malignant cancers, but they can also be effectively treated because PSC are exceptionally sensitive to the chemotherapeutic agent, Cis-Platinum (Einhorn and Donohue, 1977, Oosterhuis et al., 1984, Einhorn et al., 1981) as part of a standard treatment that also includes Bleomycin and Etoposide (Williams et al 1987). Although some PSC do produce teratocarcinomas in which undifferentiated PSC can be recognized histologically, or by outgrowths of PSC from explanted tumors (Andrews et al., 2005), many of the xenograft tumors derived from PSC are better classified as teratomas (Allison et al., 2018). It might be anticipated that variant PSC carrying mutations that enhance their proliferative potential and, perhaps, reduce their propensity to differentiate would be more likely to generate teratocarcinomas. Certainly, aneuploid PSC can produce teratocarcinomas (Andrews et al., 2005). Further, the transcriptomes of ESC carrying an extra copy of chromosome 12 clustered more closely with EC cells from germ cell tumors, which almost always exhibit a gain of the short arm of chromosome 12, while ESC with a gain of chromosome 12 were more likely to produce teratocarcinomas than the parent diploid cells from which they were derived (Ben-David et al., 2014).

On the other hand, in a recent ISCI study, albeit limited in scope, teratocarcinomas were produced by PSC without overt karyotypic abnormalities, whereas PSC with such variants, including gains of chromosome 12, produced teratomas, indicating no clear correlation between the formation of teratocarcinomas and the presence of overt karyotypic changes (Allison et al., 2018). These discrepancies point to the need for a more systematic study of the relationship between genotype and the ability of PSC to form teratocarcinomas rather than teratomas.

On the other hand, regenerative medicine applications depend upon transplantation of specific differentiated derivatives, not undifferentiated cells, so it is the possibility that genetic variants of PSC may cause a neoplastic transformation of their derivative differentiated cells that is the greater concern **(Figure 2)**. Unfortunately, there is very little direct evidence upon which to draw any definite conclusions about the extent of the risks. The somatic elements in teratomas of the laboratory mouse are almost always benign and non-tumorigenic (Damjanov and Solter, 1974) and this may be generally true of human teratomas. However, pathologists with expertise in clinical gonadal teratocarcinomas do have concerns since in the human tumors, in contrast to those of the laboratory mouse, many of the differentiated elements such as neural tubes exhibit features of immaturity that may be regarded as potentially neoplastic (Damjanov and Andrews, 2016). Certainly, secondary somatic tumors derived from primary germ cell tumors have been found clinically, although they are very rare (Mostofi and Price, 1973). Experimental, PSC-derived teratomas often also contain primitive endodermal elements, which is a further concern since yolk sac carcinoma, representing malignant primitive endoderm, is a well-known clinical form of germ cell tumors of the newborn (Cunningham et al., 2012).

Although the relationship of malignant transformation of teratoma elements to particular genetic variants has not been established, some of the common karyotypic variants occurring in human PSC are also associated with other types of somatic cancer - for example, gains of the long arm of chromosome 17 with neuroblastoma (Plantaz et al., 1997) (**BOX 2**). Further, two of the genes associated with recurrent variants in human PSC, *TP53* (Merkle et al., 2017) and *BCL2L1*, the driver gene of the chromosome 20q amplicon (see below) (Avery et al., 2013), which derive their selective advantage for PSC from their anti-apoptotic functions, are associated with many cancers (Beroukhim et al., 2010, Hainaut and Hollstein, 1999). Although the driver genes of the other common recurrent variants of human PSC have yet to be identified, they provide a selective advantage because of their specific effects on the undifferentiated PSC and it is entirely possible that their effects on specific differentiated derivatives may be quite different. However, since many of the recurrent variants involve gains or losses of large chromosomal regions, it is also possible that other 'hitch-hiker' genes linked to the driver gene may also cause effects in the differentiated derivatives, separately from the effects of the driver genes on the undifferentiated PSC themselves.

Apart from cancer, the genetic variants of PSC have the potential to cause a wide range of effects on cellular physiology that could compromise the efficacy of derivative cells used in clinical

applications, or the production of such cells, or indeed the use of PSC in research, for example into disease mechanisms. Nevertheless, there has been very little systematic consideration of these issues. They were discussed by international key opinion leaders at a meeting of the International Stem Cell Initiative (ISCI), at the Jackson Laboratory in 2016 (Andrews et al., 2017), and again at a meeting hosted by Nature in London in 2018 (Technologies, 2018), while the Japanese regulatory authorities have issued some guidelines (Research and Development Division, Health Policy Bureau, The Ministry of Health, Labour and Welfare (MHLW), http://www.nihs.go.jp/cbtp/sispsc/pdf/Eg.ver.Annex_0613-3_2016.pdf). However, there is no international consensus about potential risk assessment and the ISCI meeting in 2016 suggested the establishment of an advisory group to collate information about the common genetic variants of PSC, including any evidence of their effects on cell behaviour, and linking that information to other cancer and disease related genomic databases. Meanwhile, the Nature meeting strongly recommended that researchers clearly document any genetic variants that may have been present in cells used for particular research, so providing the data for future retrospective analysis of their potential consequences. Certainly, as a minimum, the documentation should include appropriate characterisation of the karyotype of the cells, and also assessment of the chromosome 20 amplicon, given that these represent the most commonly observed genomic changes seen in these cells.

SELECTION DRIVES THE APPEARANCE OF RECURRENT GENETIC VARIANTS

Growth advantage

Although genetic variants may be occasionally fixed when PSC cultures are passed through a population bottleneck, such as cloning, the recurrence of specific mutations within PSC populations suggests that such genetic changes endow the variant cells with a selective growth advantage. Consistent with this, the proportions of variant cells in a culture typically increase over time from when they are first detected (Draper et al., 2004, Catalina et al., 2008, Imreh et al., 2006). Similarly, in experiments designed to recapitulate the takeover of cultures by variant clones, co-mixing a small proportion of commonly occurring variants with their wild-type counterparts led to a gradually increased representation of variant cells in subsequent passages until they eventually dominated the cultures (Olariu et al., 2010, Avery et al., 2013). Commonly, a variant may be first detected when it constitutes around 5 – 10% of the cells in a culture, rising rapidly to 100% in as few as 5 passages. Based on these longitudinal evaluations, the takeover of PSC cultures by variant cells has been likened to Darwin's principle of natural selection, whereby the variant PSC that are best adapted to particular selective conditions outcompete their neighbours and populate cultures with their own progeny. The specific phenotypic features associated with genetic variants hold clues as to the selective pressures operating in PSC cultures, the reduction of which is key to minimising the appearance of genetic variants in expanding PSC populations.

In principle, genetically variant PSC could gain a selective advantage by acquiring one or several of the following features: a proliferative advantage underpinned by faster cell cycle time, a decreased rate of differentiation, or altered pattern of differentiation, or an increased rate of survival **(Figure 3)**. Indeed, a number of studies have reported that such traits typify variant cells harbouring the commonly acquired aneuploidies. For example, the growth advantage of trisomy 12 PSC was attributed mainly to their significantly reduced cell cycle time, although the variant cells also displayed an increased resistance to apoptosis and a reduced tendency for differentiation (Ben-David et al., 2014).

With regard to the reduced propensity for differentiation, no studies have so far reported a total block of variant ESC or iPSC to differentiation, although nullipotent EC cells are well known in the context of testicular germ cell tumors (Andrews et al., 1980, Andrews et al., 1982). Rather, either a reduced differentiation capacity (Fazeli et al., 2011) or a delayed differentiation dynamic (Werbowetski-Ogilvie et al., 2009) compared with wild-type cells has been observed. In some instances, genetically variant PSC appeared to yield alternative cell types to wild-type cells exposed to the same set of differentiation conditions. For example, the same differentiation protocol applied to wild-type PSC and variants with a gain of the long arm of chromosome 17 resulted in mesodiencephalic dopaminergic neurons or dorsal telencephalic neurons, respectively (Lee et al., 2015). Given that this gain entails amplification of a large chromosomal region and, hence, increased expression of most of the genes in that region (Enver et al., 2005), it is easy to envision that such extensively altered gene and protein expression profiles could include changes that skew the differentiation trajectory of cells. In this case, the skewed differentiation was attributed to an increased expression of *WNT3* and *WNT9B* genes localized in the amplified part of chromosome 17 (Lee et al., 2015). In another case, it has been reported that BCL-XL over expression perturbs SMAD and TGFβ signalling in PSC with the chromosome 20q11.21 gain, resulting in impaired neurectoderm differentiation (Markouli et al., 2019). Although the altered propensity for differentiation of variant cells may be a mere consequence of hitch-hiker genes rather than the driver of their growth advantage, the converse may also hold true if the differentiation process itself exerts selection on the differentiating cells. For example, in one study, cardiac differentiation favoured cells with a gain of the long arm of chromosome 20 (Laurent et al., 2011) whereas in another report Merkle et al (Merkle et al., 2017) noted an enrichment of mutant *TP53* cells upon PSC differentiation. In both cases, a variant PSC population was already present in the starting cultures prior to differentiation, but it is also possible that variant cells may arise and be selected during the differentiation process itself.

Although faster cell cycle and altered differentiation have been associated with some of the recurrent variants, resistance to apoptosis seems to be a frequent feature of variants commonly detected in PSC cultures. This is, perhaps, not surprising given that marked sensitivity to apoptosis represents one of the notable features of early-passage diploid PSC. Excessive cell death is particularly prominent when PSC are grown at a low cell density (Ohgushi et al., 2010), a condition under which

single PSC are confronted with a series of bottlenecks preventing their clonal growth (Barbaric et al., 2014). At the molecular level, propensity for apoptosis has been explained by a low apoptotic threshold of PSC, governed by low expression levels of anti-apoptotic proteins and high expression levels of pro-apoptotic proteins (Liu et al., 2013). In addition to preferential expression of pro-apoptotic factors, PSC store a constitutively active pro-apoptotic factor BAX in the Golgi (Dumitru et al., 2012). This effectively primes PSC for a rapid apoptotic response to appropriate cues. Apart from hampering the efficient scale up of PSC, the severe reduction in cell numbers during culture clearly creates conditions for selection of genetically variant cells capable of blunting the apoptotic pathways (Avery et al., 2013, Merkle et al., 2017).

The emergence of variant cells in PSC cultures inevitably entails interactions of variants with their wild-type counterparts, as the two populations share their environment and some of their cell-cell contacts. The nature of these interactions can determine the fate of wild-type cells in a non-cell autonomous manner, thereby impacting on the dynamics of the variant's overtake of cultures (**Figure 3**). Some of the commonly occurring PSC variants were shown to suppress the growth of wild-type populations by inducing apoptosis in their neighbouring wild-type cells (Price et al., 2019), in a manner similar to the phenomenon of cell competition described in other model systems (Bowling et al., 2019). In PSC cultures, a differential sensitivity of wild-type and variant PSC to mechanical pressures imposed by cell crowding allowed variants to effectively eliminate wild-type cells from mixed cultures, therefore enhancing the ability of variants to rapidly achieve the clonal dominance (Price et al., 2019). Therefore, consideration of cell interactions, in addition to cell autonomous mechanisms, is needed in developing effective strategies for prevention of growth supremacy of variant cells.

Driver genes

The simplest working hypothesis to account for the recurrent selection of a particular chromosomal variant is that it is the altered expression of a single 'driver' gene located in the variant region that provides a growth advantage by altering a cell's behaviour in response to proliferation, differentiation or cell death cues (**Figure 3**). It is, of course, possible that interaction of multiple linked genes in a particular chromosomal rearrangement, or indeed alterations to the chromatin architecture itself, may be responsible. Nevertheless, most studies have focused on seeking a single driver gene.

Often the size of the genomic region affected is too large to home in on a likely candidate, but in the case of amplifications affecting chromosome 20, a common minimal amplicon of 0.55Mb was identified in the pericentromeric region of the long arm in all reported examples (Amps et al., 2011). Within this minimal amplicon, containing only thirteen annotated genes, *BCL2L1* was a likely candidate driver gene as its anti-apoptotic splice variant, BCL-XL, is expressed in human PSC (Amps et al., 2011). Experiments in which cells carrying a gain chromosome 20, or that had been transfected with a *BCL2L1*-over expressing vector, were mixed with diploid cells, confirmed that

BCL2L1 and its BCL-XL product was indeed the driver providing a selective growth advantage by blocking apoptosis (Nguyen et al., 2013, Avery et al., 2013).

Like chromosome 20, a common minimal amplicon has also been identified on the long arm of chromosome 1 (Baker et al., 2016)(E. McIntire et al, International Society for Stem Cell Research Meeting abstract). A likely candidate driver gene located in this region is *MDM4,* which regulates p53 by suppressing its response to cellular stresses and increasing the threshold to apoptosis (Haupt et al., 2019). Since recurrent dominant negative mutations of *TP53* provide a growth advantage to human PSC (Merkle et al., 2017) it is likely that dysregulation of other genes, such as *MDM4*, that affect apoptosis through p53 would confer a similar growth advantage. On the long arm of chromosome 17, another anti-apoptotic gene, *SURVIVIN (BIRC5)*, encoded in the chromosome 17q25.3 region, has been proposed since its inhibition leads to apoptosis of human PSC and cancer cells (Blum et al., 2009, Mesri et al., 2001, Ma et al., 2006, Yang et al., 2004). On the other hand, other candidate genes encoded on chromosome 17 include *WNT3* and *WNT9B*, suggested by their involvement in the altered patterns of differentiation of cells carrying a gain of the long arm of chromosome 17 (Lee et al., 2015).

Interest in gains of chromosome 12 has a long history since testicular germ cell tumors almost always have a gain of the short arm, mostly as an isochromosome (Atkin and Baker, 1982), or more rarely as an interstitial amplification (Rodriguez et al., 2003, Korkola et al., 2006). However, there is no definitive evidence to identify the specific driver gene, either for the progression of germ cell tumors, or for the appearance of variant human PSC with a gain of chromosome 12. An obvious candidate driver gene on the short arm of chromosome 12 is *NANOG*, given its central role in maintaining pluripotency, and that its over-expression inhibits differentiation (Chambers et al., 2007). Also, over expression of NANOG does allow human ESC to efficiently form colonies at low density, which is normally associated with extensive apoptosis, perhaps mediated by downregulating LECTIN1, which normally promotes apoptosis, and upregulating HSPA1A, which inhibits apoptosis (Darr et al., 2006). Indeed, *NANOG* is located in a minimal amplicon that has been identified in germ cell tumors, chromosome 12p13.31, but so are two other genes, *DPPA3* and *GDF3*, that also may affect the behaviour of human PSC (Korkola et al., 2006). However, a different minimal amplicon has also been reported in human germ cell tumors, at 12p11.2–p12.1, in which a number of other genes have been highlighted, such as the oncogene, *KRAS* (Rodriguez et al., 2003).

Although less frequent, deletions may promote enhanced survival through copy number loss of pro-apoptotic genes. The BCL-2 apoptotic pathway is controlled by interactions between pro- and anti-apoptotic protein family members. Human ESC show elevated expression of the pro-apoptotic genes *NOXA, BIK, BIM, BMF* and *PUMA*, which may contribute to their low apoptotic threshold (Madden et al., 2011, Liu et al., 2013, Dumitru et al., 2012). The two of these most highly expressed in human PSC, *NOXA* and *BIK*, are located in chromosomal regions, 18q21.32 and 22q13.2, that do undergo recurrent deletion. Deletion of *NOXA* by genetic manipulation decreases the sensitivity of

human PSC to mitotic errors, thereby increasing the survival of aneuploidy cells (Zhang et al., 2019), and improves survival during cell dissociation, similar to the overexpression of the anti-apoptotic proteins, BCL-2 and BCL-XL (Ohgushi et al., 2010, Ardehali et al., 2011).

ACQUISITION OF MUTATIONS

Mutation Rate in PSC

Whereas the mechanisms by which genetic variants offer cells a selective growth advantage are relatively easy to assess and have been extensively studied, addressing the mechanisms that drive the appearance of the variants in the first instance is more problematic: Mutations occur stochastically and at low frequency in single cells within much larger populations, so that by the time they become detectable the frequency of mutation may have been grossly distorted by the effects of selection. To overcome this problem, we recently adopted a clonogenic strategy in which a single cell was isolated and allowed to expand as a clonal colony for a fixed time, after which the clone was subcloned, with about 20 subclones being isolated and, after expansion, subjected to whole genome sequencing (Thompson et al., 2020). Using this approach, in which most of the mutants that arose were in genes and locations unlikely to result in growth advantage or disadvantage, we estimated the mutation rate of two human, clinical grade ESC lines, MShef4 and MShef11, as 0.37 $x10^{-9}$ and 0.28$x10^{-9}$ SNVs per base pair, per day, respectively, equating to approximately 0.30 $x10^{-9}$ and 0.23$x10^{-9}$ SNVs per cell division, respectively, given that the cell cycle time of human PSC, in our experience, approximates 20 hours (+/- 2 hours) (Barbaric et al., 2014). This rate was not affected by the use of the Rho associated coiled coil containing protein kinase (ROCK) inhibitor, Y-27632, commonly used in human PSC culture (Watanabe et al., 2007) The frequency of INDELS was 10-fold lower. These low rates are comparable with another study of human iPSC in which the mutation rate was estimated to be 0.18 x 10^{-9} SNV per base-pair, per cell division which was considerably lower than in the endothelial progenitor cells (Rouhani et al., 2016). These mutation rates in human PSC contrast with an estimated rate of 2.66 x 10^{-9} mutations per base pair, per mitosis in somatic cells (Milholland et al., 2017). In another more limited study of a human ESC line, a slightly higher mutation rate of 1 x 10^{-9} SNV per base-pair, per cell division, but again this was much lower than an estimate of a corresponding somatic cell in the same study (Kuijk et al., 2018), while in a study of a single locus, *Aprt*, in mouse ESC, the mutation rate was estimated to be 10 fold lower than in corresponding somatic cells (Cervantes et al., 2002). These low rates are consistent with the infrequency of recurrent point mutations observed in PSC lines: for example Merkle et al (2017) (Merkle et al., 2017) only observed mutations in TP53 in five out of 140 human PSC lines.

In our study of the MShef4 and MShef11 human ESC lines (Thompson et al., 2020), the mutation rate was similar across all chromosomes, with no obvious hotspots, with the exception of a slightly raised rate on the X chromosome, which might have been a consequence of both lines being male. Nevertheless, the mutation rate was significantly higher in intergenic regions than in exons and

introns, suggesting an influence of chromatin structure on mutation. Further, the predominant mutation signatures that we detected were consistent with oxidative damage being the predominant cause of mutation and, indeed, the mutation rate for both SNV and INDELS was reduced by about 50% when the cells were maintained under low (5%) oxygen atmospheres.

DNA Replication Stress and Mitotic Errors

While many SNV in PSC, as in other cultured cells (Petljak et al., 2019, Kucab et al., 2019, Viel et al., 2017), are caused by misincorporation of bases due to oxidative stress, the relatively rapid cell cycle of PSC might also expose them to high levels of DNA replication stress, characterised by reduced rates of DNA replication together with stalling and collapse of replication forks (Bartkova et al., 2005, Gorgoulis et al., 2005). Errors in the repair of resulting double stranded DNA breaks could then lead to chromosomal rearrangements (Cannan and Pederson, 2016). Self-renewal of human PSC is characterised by an abbreviated G1 phase that bypasses the RB1-E2F checkpoint due to the high expression of cyclin D2 and its CDK4 partner together with the constitutive expression of cyclin E, which together maintain RB1 in a hyperphosphorylated and inactive state (Becker et al., 2006, Becker et al., 2010, Filipczyk et al., 2007). Using DNA fibre assays we have recently found that, in comparison to isogenic somatic cells, human PSC do exhibit the features of DNA replication stress, including slower DNA replication speeds with evidence of stalled replication forks, and replication initiating from quiescent replication origins (**Figure 4**) (Halliwell et al., 2019). In parallel we also observed more extensive replication-associated DNA damage in the PSC compared to somatic cells, as has also been reported by others (Simara et al., 2017, Vallabhaneni et al., 2018).

A similar situation pertains in many cancers where cyclin E is frequently over-expressed and RB1-E2F is constitutively activated (Akli and Keyomarsi, 2003). One of the consequences of this is replication stress, double stranded breaks and genetic instability (Bester et al., 2011, Burrell et al., 2013, Frame et al., 2006, Pickering and Kowalik, 2006). In mouse ESC, also, molecular hallmarks of replication stress are almost identical to those observed when oncogenes, such as cyclin E, are dysregulated in somatic cells (Ahuja et al., 2016), suggesting that atypical cell cycle control with consequent susceptibility to DNA replication stress and genomic damage in PSC parallels the oncogene-induced DNA damage model for cancer development and progression (Halazonetis et al., 2008).

Replication stress induced from oncogene expression can lead to nucleotide deficiency and collision of replication forks with transcription complexes (Jones et al., 2013, Bester et al., 2011). Supplementing cancer cells or primary cell lines that overexpress oncogenes, such as cyclin E, with nucleosides has been found to alleviate replication stress and its associated DNA damage and genetic instability in these cases (Bester et al., 2011, Burrell et al., 2013). In a similar manner, we

have recently found that exogenous nucleosides increase the rate of replication fork progression and decrease DNA damage in human PSC cultures (**Figure 4**) (Halliwell et al., 2019).

While chromosomal non-dysjunction and numerical instabilities may be the product of merotelic kinetochore attachment, in which the microtubules from both poles bind to the same sister chromatid, leading to lagging and potential mis-segregation of chromosomes (Cimini et al., 2001), the persistence of DNA replication defects from S phase into mitosis can also result in the formation of mitotic errors that are a source of chromosomal instabilities (Burrell et al., 2013). Under-replicated regions can interlink sister chromatids during segregation forming anaphase bridges that are prone to breakage forming double stranded breaks (Chan et al., 2009). Often, to prevent anaphase bridges, nucleases cleave the DNA that again generates double stranded breaks (Naim et al., 2013). Further, the condensation of chromosomes that harbour replication intermediates are particularly prone to breakage (Lukas et al., 2011). These double stranded breaks that result from replication intermediates in mitosis are the substrates for genetic instability caused by error induced repair.

By fluorescently labelling human PSC with histone H2B-mCherry it was observed that 30% of mitoses were abnormal including a high proportion with lagging chromosomes and anaphase bridges (Zhang et al., 2019), a level substantially higher than that observed in somatic cell lines (Lamm et al., 2016). In comparison to somatic cell lines, diploid human PSC show condensation defects that result in partially condensed and entangled chromosomes (Lamm et al., 2016). Supplementing cultures with exogenous nucleosides alleviated replication stress and decreased the frequency of mitotic errors, providing further evidence that these are linked in human PSC (Halliwell et al., 2019), as well as providing an approach to reducing their appearance in PSC cultures. However, it should be noted that the continued occurrence of mitotic errors, even with the addition of nucleosides suggests that there are other factors driving their occurrence.

Response to genomic damage

Human PSC deploy a number of mechanisms to minimise the effective mutation rate that otherwise might be anticipated from their high susceptibility to DNA damage. Genes involved in various repair pathways show increased expression compared to somatic cells (Maynard et al., 2008, Momcilović et al., 2009), and nucleotide excision repair, base excision repair, and the resolution of inter-strand crosslinks caused by ionising radiation all have all been reported to be faster in human PSC than somatic cell lines (Luo et al., 2012, Hyka-Nouspikel et al., 2012, Maynard et al., 2008). PSC also tend to repair double strand break using homologous recombination, which is prone to less errors than non-homologous end joining (Adams et al., 2010a), although they do also utilise a higher fidelity system of non-homologous end joining that is independent of DNA-PKc and ATM (Adams et al., 2010b). Further, in response to the formation of reactive oxygen species as a by-product of respiration, and consequent oxidative stress, PSC express higher levels of SOD2 and GPX2 anti-oxidant enzymes compared to differentiated lines (Saretzki et al., 2008).

Nevertheless, human PSC generally activate apoptosis when exposed to lower doses of genotoxic insults than do somatic cells, suggesting that a low apoptotic threshold is the key element in their response to genomic damage. After human PSC are exposed to ultraviolet C radiation to induce nucleotide base adducts or DNA breaks they respond with extensive apoptosis even at mild doses that have little effect on somatic cell lines (Hyka-Nouspikel et al., 2012, Luo et al., 2012, Simara et al., 2017). Similarly, the treatment of human PSC with cis-platinum or thymidine to initiate replication block (Desmarais et al., 2012, Desmarais et al., 2016), or with nocadazole to induce mitotic block (Zhang et al., 2019) also elicits an extensive apoptotic response in contrast to the response by somatic cells, while PSC also efficiently activate apoptosis in response to oxidative stress (Saretzki et al., 2008). The particular sensitivity of embryonal carcinoma cells, the malignant PSC of teratocarcinomas, to drugs such as cis-platinum (Oosterhuis et al., 1984, Einhorn and Donohue, 1977), a DNA cross linking agent, makes germ cell tumors one of the most treatable forms of solid cancer, most likely reflecting this particular low apoptotic threshold.

An atypical cell cycle checkpoint control mechanism most likely underlies the low apoptotic threshold of human PSC. In response to DNA damage, human PSC fail to activate p21, which is normally required to execute the G1/S checkpoint, providing less time for repair before apoptosis is initiated in a p53 dependant manner (Hyka-Nouspikel et al., 2012, Hong and Stambrook, 2004, Momcilović et al., 2009). Further, in response to DNA replication stress caused by high levels of thymidine, or the presence of cis-platinum, human PSC, unlike somatic cells, do not activate ATR-CHK1, while foci of RPA, which binds to single stranded DNA at stalled replication forks, are not formed: instead the cells commit to apoptosis (Desmarais et al., 2012, Desmarais et al., 2016). Human PSC also undergo extensive apoptosis in response to mitotic stress which may safeguard the genome from abnormal mitosis by clearing the effected cell from the cell pool (Zhang et al., 2019). Collectively, these studies support a model in which genomic stability, and the particularly low observed mutation rate of PSC is primarily maintained by a low apoptotic threshold. Consequently, blocking apoptosis seems to be the most likely mechanism that provides selective growth advantage for the common genetic variants found in human PSC: the two driver genes so far identified, *TP53* and *BCL2L1*, both act to inhibit apoptosis, while other proposed candidates, MDM4 and SURVIVIN, are also anti-apoptotic.

This low apoptotic threshold of human PSC may reflect their relationship to the early embryo in which the need for rapid cell doublings is accomplished by the lack of cell cycle checkpoints, rendering the cells particularly susceptible to errors in DNA synthesis and mitosis, which could be catastrophic for subsequent embryonic development. Indeed, almost half of human embryos fail to survive due to chromosomal instability, which does not seem to be a mere artefact of *in vitro* fertilisation (van Echten-Arends et al., 2011, Munné et al., 2019, Starostik et al., 2020). It has been observed that the mosaic embryos that survive to the blastocyst stage undergo “genetic normalization” when cultured under routine IVF conditions (Brezina et al., 2011). The mechanism of genetic normalization

is still widely debated although, in the mouse, activation of apoptosis during the later pre-implantation stages may allow for the removal of aneuploid cells from the developing embryo (Kops et al., 2004, Bolton et al., 2016). This model is supported by observations that the proportion of aneuploidy in the inner cell mass is reduced, whereas in the trophectoderm it is enriched as development proceeds (Hardy, 1997). However, this is still widely debated as apoptosis is a feature of all embryos, and may be a mechanism for maintaining cellular homeostasis regardless of their genomic state (Haouzi and Hamamah, 2009).

Another context in which the apoptotic response of human PSC to DSB induction is of central importance, is the process of genome editing. The rising prominence of genome editing technologies, in particular CRISPR/Cas9-based methods, has fuelled efforts aimed at, for example, correcting germline mutations in PSC to allow autologous cell therapy, or removing HLA antigens to reduce the need for immunosuppressants in transplanted patients. Crucially, as gene editing relies on the induction of DSB by nucleases, the edited PSC undergo high levels of apoptosis in comparison to their unedited counterparts (Ihry et al., 2018). The rate of cell death was shown to be similar between different, edited PSC, regardless of whether the targeted gene was expressed in PSC or whether it was non-expressed and dispensable for PSC maintenance. This observation supports the view that the induction of DSB during the gene editing process commits PSC to apoptosis (Ihry et al., 2018). Mechanistically, DSB induction by Cas9 was shown to trigger differential gene expression in edited cells, most notably by promoting the TP53 transcriptional response (Ihry et al., 2018). In line with the importance of TP53 activation during the genome editing process, performing the PSC genome editing in cells with genetically inactivated *TP53* reduced the levels of cell death and improved the efficiency of PSC genome editing (Ihry et al., 2018). While genetic inactivation of TP53 is deemed too risky for editing of cells destined for clinical use, transient TP53 inactivation has been proposed as a possible alternative (Schiroli et al., 2019). Further work will need to carefully address this possibility, to ensure that the transient TP53 inactivation does not inadvertently select for *TP53* genetic mutants. Relevant to this notion is the recent data demonstrating the emergence of *TP53*-inactivating mutations in cancer cell lines during the CRISPR/Cas9 genome editing process (Enache et al., 2020).

CONCLUSIONS

Efforts to collect and catalogue genetic variation in PSC over the last two decades demonstrated that particular variants do arise in cultures and are sometimes difficult to detect because of the limitation of sensitivity of detection methods. Nonetheless, it is reassuring that the rate of mutation in PSC cultures is low compared with somatic cells. Indeed, based on the data from large-scale retrospective studies, such as the ISCI (Amps et al., 2011), and on the direct measurements of the mutation rates in PSC (Thompson et al., 2020), there is no evidence to suggest that PSC genomes are particularly unstable. Rather, the clonal expansion of genetic variants against the backdrop of

low mutation rates, can be explained by the effect of selective pressures operating in PSC cultures. Optimizing culture conditions and protocols to minimize the growth advantages of the common variants is, therefore, a key route to maintaining the genetic integrity of PSC lines. However, the predominant selective force dominating PSC cultures appears to be a high rate of apoptosis (Dumitru et al., 2012, Barbaric et al., 2014). Apoptosis seems to be a default fate choice of PSC in many different scenarios, including a response of cells to genome damage or mitotic stress (Desmarais et al., 2012, Desmarais et al., 2016, Dumitru et al., 2012, Zhang et al., 2019), most likely reflecting its function of maintaining the genetic integrity of the early embryo. Consequently, optimising culture conditions should entail removing the apoptotic stimuli, but not blocking apoptosis *per se* which would be counterproductive.

Armed with a knowledge of recurrent karyotypic and sequence changes, our attention now needs to turn to finding ways of minimizing their occurrence by lowering the genome damage and reducing the selective pressures. In that respect, the observations that mutation rates can be decreased by growing cells under low oxygen (Thompson et al., 2020) and that replication-stress induced genome damage can be alleviated by addition of exogenous nucleosides (Halliwell et al., 2019) provide foundations for optimised culture conditions of PSC. Further work should also address the contribution of epigenetic variants to aberrant PSC phenotypes, as the understanding of epigenetic variation in PSC cultures remains limited.

Finally, the field awaits deciphering of the functional consequences of the karyotype and sequence changes on PSC traits and on the behaviour of their differentiated derivatives. Interpreting the role of specific variants is complicated by the fact that their consequences are likely to be context-dependent. For example, a mutation in a gene expressed specifically in an endodermal lineage may have little impact on the clinical application of neuronal cells. To aid these analyses, ISCI is proposing an international study group to collate and monitor evidence of genetic variants in PSC and their potential consequences (Andrews et al., 2017). However, it should be noted that the success of this approach requires a concerted effort within the field to perform routine monitoring and report the presence of genetic variants, thereby allowing retrospective analyses of their effects. We envisage that these initiatives, in a synergy with cancer genome efforts, would provide a rational strategy to assess the potential risk of different mutations, a necessary requirement for routine, safe clinical implementation of cellular therapies.

REFERENCES

ADAMS, B. R., GOLDING, S. E., RAO, R. R. & VALERIE, K. 2010a. Dynamic dependence on ATR and ATM for double-strand break repair in human embryonic stem cells and neural descendants. *PLoS One,* 5**,** e10001.

ADAMS, B. R., HAWKINS, A. J., POVIRK, L. F. & VALERIE, K. 2010b. ATM-independent, high-fidelity nonhomologous end joining predominates in human embryonic stem cells. *Aging (Albany NY),* 2**,** 582-96.

615 AHUJA, A. K., JODKOWSKA, K., TELONI, F., BIZARD, A. H., ZELLWEGER, R., HERRADOR,
616 R., ORTEGA, S., HICKSON, I. D., ALTMEYER, M., MENDEZ, J. & LOPES, M. 2016. A
617 short G1 phase imposes constitutive replication stress and fork remodelling in mouse
618 embryonic stem cells. *Nat Commun,* 7**,** 10660.
619 AKLI, S. & KEYOMARSI, K. 2003. Cyclin E and its low molecular weight forms in human cancer
620 and as targets for cancer therapy. *Cancer Biol Ther,* 2**,** S38-47.
621 ALLISON, T. F., ANDREWS, P. W., AVIOR, Y., BARBARIC, I., BENVENISTY, N., BOCK, C.,
622 BREHM, J., BRÜSTLE, O., DAMJANOV, I., ELEFANTY, A., FELKNE, D., GOKHALE,
623 P. J., HALBRITTER, F., HEALY, L. E., HU, T. X., KNOWLES, B. B., LORING, J. F.,
624 LUDWIG, T. E., MAYBERRY, R., MICALLEF, S., MOHAMED, J. S., MÜLLER, F.-J.,
625 MUMMERY, C. L., NAKATSUJI, N., NG, E. S., OH, S. K. W., O'SHEA, O., PERA, M. F.,
626 REUBINOFF, B., ROBSON, P., ROSSANT, J., SCHULDT, B. M., SOLTER, D., SOURRIS,
627 K., STACEY, G., STANLEY, E. G., SUEMORI, H., TAKAHASHI, K. & YAMANAKA, S.
628 2018. Assessment of established techniques to determine developmental and malignant
629 potential of human pluripotent stem cells. *Nat Commun,* 9**,** 1925.
630 AMPS, K., ANDREWS, P. W., ANYFANTIS, G., ARMSTRONG, L., AVERY, S., BAHARVAND,
631 H., BAKER, J., BAKER, D., MUNOZ, M. B., BEIL, S., BENVENISTY, N., BEN-YOSEF,
632 D., BIANCOTTI, J. C., BOSMAN, A., BRENA, R. M., BRISON, D., CAISANDER, G.,
633 CAMARASA, M. V., CHEN, J., CHIAO, E., CHOI, Y. M., CHOO, A. B., COLLINS, D.,
634 COLMAN, A., CROOK, J. M., DALEY, G. Q., DALTON, A., DE SOUSA, P. A.,
635 DENNING, C., DOWNIE, J., DVORAK, P., MONTGOMERY, K. D., FEKI, A., FORD, A.,
636 FOX, V., FRAGA, A. M., FRUMKIN, T., GE, L., GOKHALE, P. J., GOLAN-LEV, T.,
637 GOURABI, H., GROPP, M., LU, G., HAMPL, A., HARRON, K., HEALY, L., HERATH,
638 W., HOLM, F., HOVATTA, O., HYLLNER, J., INAMDAR, M. S., IRWANTO, A. K., ISHII,
639 T., JACONI, M., JIN, Y., KIMBER, S., KISELEV, S., KNOWLES, B. B., KOPPER, O.,
640 KUKHARENKO, V., KULIEV, A., LAGARKOVA, M. A., LAIRD, P. W., LAKO, M.,
641 LASLETT, A. L., LAVON, N., LEE, D. R., LEE, J. E., LI, C., LIM, L. S., LUDWIG, T. E.,
642 MA, Y., MALTBY, E., MATEIZEL, I., MAYSHAR, Y., MILEIKOVSKY, M., MINGER,
643 S. L., MIYAZAKI, T., MOON, S. Y., MOORE, H., MUMMERY, C., NAGY, A.,
644 NAKATSUJI, N., NARWANI, K., OH, S. K., OLSON, C., OTONKOSKI, T., PAN, F.,
645 PARK, I. H., PELLS, S., PERA, M. F., PEREIRA, L. V., QI, O., RAJ, G. S., REUBINOFF,
646 B., ROBINS, A., ROBSON, P., ROSSANT, J., SALEKDEH, G. H., SCHULZ, T. C., et al.
647 2011. Screening ethnically diverse human embryonic stem cells identifies a chromosome 20
648 minimal amplicon conferring growth advantage. *Nat Biotechnol,* 29**,** 1132-44.
649 ANDREWS, P. W. 2002. From teratocarcinomas to embryonic stem cells. *Philos Trans R Soc Lond*
650 *B Biol Sci,* 357**,** 405-17.
651 ANDREWS, P. W., BEN-DAVID, U., BENVENISTY, N., COFFEY, P., EGGAN, K., KNOWLES,
652 B. B., NAGY, A., PERA, M., REUBINOFF, B., RUGG-GUNN, P. J. & STACEY, G. N.
653 2017. Assessing the Safety of Human Pluripotent Stem Cells and Their Derivatives for
654 Clinical Applications. *Stem Cell Reports,* 9**,** 1-4.
655 ANDREWS, P. W., BRONSON, D. L., BENHAM, F., STRICKLAND, S. & KNOWLES, B. B.
656 1980. A comparative study of eight cell lines derived from human testicular teratocarcinoma.
657 *Int J Cancer,* 26**,** 269-80.
658 ANDREWS, P. W., GOODFELLOW, P. N., SHEVINSKY, L. H., BRONSON, D. L. & KNOWLES,
659 B. B. 1982. Cell-surface antigens of a clonal human embryonal carcinoma cell line:
660 morphological and antigenic differentiation in culture. *Int J Cancer,* 29**,** 523-31.
661 ANDREWS, P. W., MATIN, M. M., BAHRAMI, A. R., DAMJANOV, I., GOKHALE, P. &
662 DRAPER, J. S. 2005. Embryonic stem (ES) cells and embryonal carcinoma (EC) cells:
663 opposite sides of the same coin. *Biochem Soc Trans,* 33**,** 1526-30.
664 ARDEHALI, R., INLAY, M. A., ALI, S. R., TANG, C., DRUKKER, M. & WEISSMAN, I. L. 2011.
665 Overexpression of BCL2 enhances survival of human embryonic stem cells during stress and
666 obviates the requirement for serum factors. *Proc Natl Acad Sci U S A,* 108**,** 3282-7.

ASSOU, S., GIRAULT, N., PLINET, M., BOUCKENHEIMER, J., SANSAC, C., COMBE, M., MIANNÉ, J., BOURGUIGNON, C., FIELDES, M., AHMED, E., COMMES, T., BOUREUX, A., LEMAÎTRE, J.-M. & DE VOS, J. 2020. Recurrent Genetic Abnormalities in Human Pluripotent Stem Cells: Definition and Routine Detection in Culture Supernatant by Targeted Droplet Digital PCR. *Stem Cell Reports,* 14**,** 1-8.

ATKIN, N. B. & BAKER, M. C. 1982. Specific chromosome change, i(12p), in testicular tumours? *Lancet,* 2**,** 1349.

AVERY, S., HIRST, A. J., BAKER, D., LIM, C. Y., ALAGARATNAM, S., SKOTHEIM, R. I., LOTHE, R. A., PERA, M. F., COLMAN, A., ROBSON, P., ANDREWS, P. W. & KNOWLES, B. B. 2013. BCL-XL mediates the strong selective advantage of a 20q11.21 amplification commonly found in human embryonic stem cell cultures. *Stem Cell Reports,* 1**,** 379-86.

AVIOR, Y., EGGAN, K. & BENVENISTY, N. 2019. Cancer-Related Mutations Identified in Primed and Naive Human Pluripotent Stem Cells. *Cell Stem Cell,* 25**,** 456-461.

BAKER, D., HIRST, A. J., GOKHALE, P. J., JUAREZ, M. A., WILLIAMS, S., WHEELER, M., BEAN, K., ALLISON, T. F., MOORE, H. D., ANDREWS, P. W. & BARBARIC, I. 2016. Detecting Genetic Mosaicism in Cultures of Human Pluripotent Stem Cells. *Stem Cell Reports,* 7**,** 998-1012.

BAKER, D. E., HARRISON, N. J., MALTBY, E., SMITH, K., MOORE, H. D., SHAW, P. J., HEATH, P. R., HOLDEN, H. & ANDREWS, P. W. 2007. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. *Nat Biotechnol,* 25**,** 207-15.

BARBARIC, I., BIGA, V., GOKHALE, P. J., JONES, M., STAVISH, D., GLEN, A., COCA, D. & ANDREWS, P. W. 2014. Time-lapse analysis of human embryonic stem cells reveals multiple bottlenecks restricting colony formation and their relief upon culture adaptation. *Stem Cell Reports,* 3**,** 142-55.

BARKER, R. A., PARMAR, M., STUDER, L. & TAKAHASHI, J. 2017. Human Trials of Stem Cell-Derived Dopamine Neurons for Parkinson's Disease: Dawn of a New Era. *Cell Stem Cell,* 21**,** 569-573.

BARTKOVA, J., HOREJSÍ, Z., KOED, K., KRÄMER, A., TORT, F., ZIEGER, K., GULDBERG, P., SEHESTED, M., NESLAND, J. M., LUKAS, C., ØRNTOFT, T., LUKAS, J. & BARTEK, J. 2005. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature,* 434**,** 864-70.

BECKER, K. A., GHULE, P. N., LIAN, J. B., STEIN, J. L., VAN WIJNEN, A. J. & STEIN, G. S. 2010. Cyclin D2 and the CDK substrate p220(NPAT) are required for self-renewal of human embryonic stem cells. *J Cell Physiol,* 222**,** 456-64.

BECKER, K. A., GHULE, P. N., THERRIEN, J. A., LIAN, J. B., STEIN, J. L., VAN WIJNEN, A. J. & STEIN, G. S. 2006. Self-renewal of human embryonic stem cells is supported by a shortened G1 cell cycle phase. *J Cell Physiol,* 209**,** 883-93.

BEN-DAVID, U., ARAD, G., WEISSBEIN, U., MANDEFRO, B., MAIMON, A., GOLAN-LEV, T., NARWANI, K., CLARK, A. T., ANDREWS, P. W., BENVENISTY, N. & CARLOS BIANCOTTI, J. 2014. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat Commun,* 5**,** 4825.

BEROUKHIM, R., MERMEL, C. H., PORTER, D., WEI, G., RAYCHAUDHURI, S., DONOVAN, J., BARRETINA, J., BOEHM, J. S., DOBSON, J., URASHIMA, M., MC HENRY, K. T., PINCHBACK, R. M., LIGON, A. H., CHO, Y. J., HAERY, L., GREULICH, H., REICH, M., WINCKLER, W., LAWRENCE, M. S., WEIR, B. A., TANAKA, K. E., CHIANG, D. Y., BASS, A. J., LOO, A., HOFFMAN, C., PRENSNER, J., LIEFELD, T., GAO, Q., YECIES, D., SIGNORETTI, S., MAHER, E., KAYE, F. J., SASAKI, H., TEPPER, J. E., FLETCHER, J. A., TABERNERO, J., BASELGA, J., TSAO, M. S., DEMICHELIS, F., RUBIN, M. A., JANNE, P. A., DALY, M. J., NUCERA, C., LEVINE, R. L., EBERT, B. L., GABRIEL, S., RUSTGI, A. K., ANTONESCU, C. R., LADANYI, M., LETAI, A., GARRAWAY, L. A., LODA, M., BEER, D. G., TRUE, L. D., OKAMOTO, A., POMEROY, S. L., SINGER, S.,

719 GOLUB, T. R., LANDER, E. S., GETZ, G., SELLERS, W. R. & MEYERSON, M. 2010. The
720 landscape of somatic copy-number alteration across human cancers. *Nature,* 463**,** 899-905.
721 BESTER, A. C., RONIGER, M., OREN, Y. S., IM, M. M., SARNI, D., CHAOAT, M., BENSIMON,
722 A., ZAMIR, G., SHEWACH, D. S. & KEREM, B. 2011. Nucleotide deficiency promotes
723 genomic instability in early stages of cancer development. *Cell,* 145**,** 435-46.
724 BLUM, B., BAR-NUR, O., GOLAN-LEV, T. & BENVENISTY, N. 2009. The anti-apoptotic gene
725 survivin contributes to teratoma formation by human embryonic stem cells. *Nat Biotechnol,*
726 27**,** 281-7.
727 BOLTON, H., GRAHAM, S. J. L., VAN DER AA, N., KUMAR, P., THEUNIS, K., FERNANDEZ
728 GALLARDO, E., VOET, T. & ZERNICKA-GOETZ, M. 2016. Mouse model of chromosome
729 mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental
730 potential. *Nat Commun,* 7**,** 11165.
731 BOWLING, S., LAWLOR, K. & RODRÍGUEZ, T. A. 2019. Cell competition: the winners and losers
732 of fitness selection. *Development,* 146.
733 BREZINA, P., BARKER, A., BENNER, A., ROSS, R., NGUYEN, K.-H., ANCHAN, R., RICHTER,
734 K., CUTTING, G. & KEARNS, W. 2011. Genetic Normalization of Differentiating
735 Aneuploid Human Embryos. *Nature Precedings*.
736 BURRELL, R. A., MCCLELLAND, S. E., ENDESFELDER, D., GROTH, P., WELLER, M. C.,
737 SHAIKH, N., DOMINGO, E., KANU, N., DEWHURST, S. M., GRONROOS, E., CHEW,
738 S. K., ROWAN, A. J., SCHENK, A., SHEFFER, M., HOWELL, M., KSCHISCHO, M.,
739 BEHRENS, A., HELLEDAY, T., BARTEK, J., TOMLINSON, I. P. & SWANTON, C. 2013.
740 Replication stress links structural and numerical cancer chromosomal instability. *Nature,* 494**,**
741 492-496.
742 CANNAN, W. J. & PEDERSON, D. S. 2016. Mechanisms and Consequences of Double-Strand DNA
743 Break Formation in Chromatin. *J Cell Physiol,* 231**,** 3-14.
744 CATALINA, P., MONTES, R., LIGERO, G., SANCHEZ, L., DE LA CUEVA, T., BUENO, C.,
745 LEONE, P. E. & MENENDEZ, P. 2008. Human ESCs predisposition to karyotypic
746 instability: Is a matter of culture adaptation or differential vulnerability among hESC lines
747 due to inherent properties? *Mol Cancer,* 7**,** 76.
748 CERVANTES, R. B., STRINGER, J. R., SHAO, C., TISCHFIELD, J. A. & STAMBROOK, P. J.
749 2002. Embryonic stem cells and somatic cells differ in mutation frequency and type. *Proc*
750 *Natl Acad Sci U S A,* 99**,** 3586-90.
751 CHAMBERS, I., SILVA, J., COLBY, D., NICHOLS, J., NIJMEIJER, B., ROBERTSON, M.,
752 VRANA, J., JONES, K., GROTEWOLD, L. & SMITH, A. 2007. Nanog safeguards
753 pluripotency and mediates germline development. *Nature,* 450**,** 1230-4.
754 CHAN, K. L., PALMAI-PALLAG, T., YING, S. & HICKSON, I. D. 2009. Replication stress induces
755 sister-chromatid bridging at fragile site loci in mitosis. *Nat Cell Biol,* 11**,** 753-60.
756 CIMINI, D., HOWELL, B., MADDOX, P., KHODJAKOV, A., DEGRASSI, F. & SALMON, E. D.
757 2001. Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic
758 mammalian tissue cells. *J Cell Biol,* 153**,** 517-27.
759 CUNNINGHAM, J. J., ULBRIGHT, T. M., PERA, M. F. & LOOIJENGA, L. H. 2012. Lessons from
760 human teratomas to guide development of safe stem cell therapies. *Nat Biotechnol,* 30**,** 849-
761 57.
762 DA CRUZ, L., FYNES, K., GEORGIADIS, O., KERBY, J., LUO, Y. H., AHMADO, A., VERNON,
763 A., DANIELS, J. T., NOMMISTE, B., HASAN, S. M., GOOLJAR, S. B., CARR, A. F.,
764 VUGLER, A., RAMSDEN, C. M., BICTASH, M., FENSTER, M., STEER, J.,
765 HARBINSON, T., WILBREY, A., TUFAIL, A., FENG, G., WHITLOCK, M., ROBSON, A.
766 G., HOLDER, G. E., SAGOO, M. S., LOUDON, P. T., WHITING, P. & COFFEY, P. J. 2018.
767 Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in
768 age-related macular degeneration. *Nat Biotechnol,* 36**,** 328-337.
769 DAMJANOV, I. & ANDREWS, P. W. 2007a. The terminology of teratocarcinomas and teratomas.
770 *Nat Biotechnol,* 25**,** 1212; discussion.

DAMJANOV, I. & ANDREWS, P. W. 2007b. The terminology of teratocarcinomas and teratomas. *Nat Biotechnol,* 25**,** 1212; discussion 1212.
DAMJANOV, I. & ANDREWS, P. W. 2016. Teratomas produced from human pluripotent stem cells xenografted into immunodeficient mice - a histopathology atlas. *Int J Dev Biol,* 60**,** 337-419.
DAMJANOV, I. & SOLTER, D. 1974. Experimental teratoma. *Curr Top Pathol,* 59**,** 69-130.
DARR, H., MAYSHAR, Y. & BENVENISTY, N. 2006. Overexpression of NANOG in human ES cells enables feeder-free growth while inducing primitive ectoderm features. *Development,* 133**,** 1193-201.
DESMARAIS, J. A., HOFFMANN, M. J., BINGHAM, G., GAGOU, M. E., MEUTH, M. & ANDREWS, P. W. 2012. Human embryonic stem cells fail to activate CHK1 and commit to apoptosis in response to DNA replication stress. *Stem Cells,* 30**,** 1385-93.
DESMARAIS, J. A., UNGER, C., DAMJANOV, I., MEUTH, M. & ANDREWS, P. 2016. Apoptosis and failure of checkpoint kinase 1 activation in human induced pluripotent stem cells under replication stress. *Stem Cell Res Ther,* 7**,** 17.
DRAPER, J. S., SMITH, K., GOKHALE, P., MOORE, H. D., MALTBY, E., JOHNSON, J., MEISNER, L., ZWAKA, T. P., THOMSON, J. A. & ANDREWS, P. W. 2004. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol,* 22**,** 53-4.
DUMITRU, R., GAMA, V., FAGAN, B. M., BOWER, J. J., SWAHARI, V., PEVNY, L. H. & DESHMUKH, M. 2012. Human embryonic stem cells have constitutively active Bax at the Golgi and are primed to undergo rapid apoptosis. *Mol Cell,* 46**,** 573-83.
EINHORN, L. H. & DONOHUE, J. 1977. Cis-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med,* 87**,** 293-8.
EINHORN, L. H., NAGY, C., FURNAS, B. & WILLIAMS, S. D. 1981. Nabilone: an effective antiemetic in patients receiving cancer chemotherapy. *J Clin Pharmacol,* 21**,** 64S-69S.
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, P. W. 2005. Cellular differentiation hierarchies in normal and culture-adapted human embryonic stem cells. *Hum Mol Genet,* 14**,** 3129-40.
FAZELI, A., LIEW, C. G., MATIN, M. M., ELLIOTT, S., JEANMEURE, L. F., WRIGHT, P. C., MOORE, H. & ANDREWS, P. W. 2011. Altered patterns of differentiation in karyotypically abnormal human embryonic stem cells. *Int J Dev Biol,* 55**,** 175-80.
FILIPCZYK, A. A., LASLETT, A. L., MUMMERY, C. & PERA, M. F. 2007. Differentiation is coupled to changes in the cell cycle regulatory apparatus of human embryonic stem cells. *Stem Cell Res,* 1**,** 45-60.
FRAME, F. M., ROGOFF, H. A., PICKERING, M. T., CRESS, W. D. & KOWALIK, T. F. 2006. E2F1 induces MRN foci formation and a cell cycle checkpoint response in human fibroblasts. *Oncogene,* 25**,** 3258-66.
GARBER, K. 2015. RIKEN suspends first clinical trial involving induced pluripotent stem cells. *Nat Biotechnol,* 33**,** 890-1.
GORGOULIS, V. G., VASSILIOU, L. V., KARAKAIDOS, P., ZACHARATOS, P., KOTSINAS, A., LILOGLOU, T., VENERE, M., DITULLIO, R. A., KASTRINAKIS, N. G., LEVY, B., KLETSAS, D., YONETA, A., HERLYN, M., KITTAS, C. & HALAZONETIS, T. D. 2005. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature,* 434**,** 907-13.
HAINAUT, P. & HOLLSTEIN, M. 1999. p53 and Human Cancer: The First Ten Thousand Mutations. *In:* VANDE WOUDE, G. F. & KLEIN, G. (eds.) *Advances in Cancer Research.* Academic Press.
HALAZONETIS, T. D., GORGOULIS, V. G. & BARTEK, J. 2008. An oncogene-induced DNA damage model for cancer development. *Science,* 319**,** 1352-5.

HAUPT, S., MEJÍA-HERNÁNDEZ, J. O., VIJAYAKUMARAN, R., KEAM, S. P. & HAUPT, Y. 2019. The long and the short of it: the MDM4 tail so far. *Journal of Molecular Cell Biology,* 11**,** 231-244.

HONG, Y. & STAMBROOK, P. J. 2004. Restoration of an absent G1 arrest and protection from apoptosis in embryonic stem cells after ionizing radiation. *Proc Natl Acad Sci U S A,* 101**,** 14443-8.

HUSSEIN, S. M., BATADA, N. N., VUORISTO, S., CHING, R. W., AUTIO, R., NÄRVÄ, E., NG, S., SOUROUR, M., HÄMÄLÄINEN, R., OLSSON, C., LUNDIN, K., MIKKOLA, M., TROKOVIC, R., PEITZ, M., BRÜSTLE, O., BAZETT-JONES, D. P., ALITALO, K., LAHESMAA, R., NAGY, A. & OTONKOSKI, T. 2011. Copy number variation and selection during reprogramming to pluripotency. *Nature,* 471**,** 58-62.

HYKA-NOUSPIKEL, N., DESMARAIS, J., GOKHALE, P. J., JONES, M., MEUTH, M., ANDREWS, P. W. & NOUSPIKEL, T. 2012. Deficient DNA damage response and cell cycle checkpoints lead to accumulation of point mutations in human embryonic stem cells. *Stem Cells,* 30**,** 1901-10.

IMREH, M. P., GERTOW, K., CEDERVALL, J., UNGER, C., HOLMBERG, K., SZÖKE, K., CSÖREGH, L., FRIED, G., DILBER, S., BLENNOW, E. & AHRLUND-RICHTER, L. 2006. In vitro culture conditions favoring selection of chromosomal abnormalities in human ES cells. *J Cell Biochem,* 99**,** 508-16.

JONES, R. M., MORTUSEWICZ, O., AFZAL, I., LORVELLEC, M., GARCÍA, P., HELLEDAY, T. & PETERMANN, E. 2013. Increased replication initiation and conflicts with transcription underlie Cyclin E-induced replication stress. *Oncogene,* 32**,** 3744-53.

KANDOTH, C., MCLELLAN, M. D., VANDIN, F., YE, K., NIU, B., LU, C., XIE, M., ZHANG, Q., MCMICHAEL, J. F., WYCZALKOWSKI, M. A., LEISERSON, M. D. M., MILLER, C. A., WELCH, J. S., WALTER, M. J., WENDL, M. C., LEY, T. J., WILSON, R. K., RAPHAEL, B. J. & DING, L. 2013. Mutational landscape and significance across 12 major cancer types. *Nature,* 502**,** 333-339.

KOPS, G. J., FOLTZ, D. R. & CLEVELAND, D. W. 2004. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc Natl Acad Sci U S A,* 101**,** 8699-704.

KORKOLA, J. E., HOULDSWORTH, J., CHADALAVADA, R. S., OLSHEN, A. B., DOBRZYNSKI, D., REUTER, V. E., BOSL, G. J. & CHAGANTI, R. S. 2006. Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with in vivo differentiation of human male germ cell tumors. *Cancer Res,* 66**,** 820-7.

KUCAB, J. E., ZOU, X., MORGANELLA, S., JOEL, M., NANDA, A. S., NAGY, E., GOMEZ, C., DEGASPERI, A., HARRIS, R., JACKSON, S. P., ARLT, V. M., PHILLIPS, D. H. & NIK-ZAINAL, S. 2019. A Compendium of Mutational Signatures of Environmental Agents. *Cell,* 177**,** 821-836.e16.

KUIJK, E., JAGER, M., ROEST, B. V. D., LOCATI, M., HOECK, A. V., KORZELIUS, J., JANSSEN, R., BESSELINK, N., BOYMANS, S., BOXTEL, R. V. & CUPPEN, E. 2018. Mutational impact of culturing human pluripotent and adult stem cells. *bioRxiv***,** 430165.

LAMM, N., BEN-DAVID, U., GOLAN-LEV, T., STORCHOVÁ, Z., BENVENISTY, N. & KEREM, B. 2016. Genomic Instability in Human Pluripotent Stem Cells Arises from Replicative Stress and Chromosome Condensation Defects. *Cell Stem Cell,* 18**,** 253-61.

LAURENT, L. C., ULITSKY, I., SLAVIN, I., TRAN, H., SCHORK, A., MOREY, R., LYNCH, C., HARNESS, J. V., LEE, S., BARRERO, M. J., KU, S., MARTYNOVA, M., SEMECHKIN, R., GALAT, V., GOTTESFELD, J., IZPISUA BELMONTE, J. C., MURRY, C., KEIRSTEAD, H. S., PARK, H. S., SCHMIDT, U., LASLETT, A. L., MULLER, F. J., NIEVERGELT, C. M., SHAMIR, R. & LORING, J. F. 2011. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. *Cell Stem Cell,* 8**,** 106-18.

874 LEE, C. T., BENDRIEM, R. M., KINDBERG, A. A., WORDEN, L. T., WILLIAMS, M. P.,
875 DRGON, T., MALLON, B. S., HARVEY, B. K., RICHIE, C. T., HAMILTON, R. S., CHEN,
876 J., ERRICO, S. L., TSAI, S. Y., UHL, G. R. & FREED, W. J. 2015. Functional consequences
877 of 17q21.31/WNT3-WNT9B amplification in hPSCs with respect to neural differentiation.
878 *Cell Rep,* 10**,** 616-32.
879 LEFORT, N., FEYEUX, M., BAS, C., FÉRAUD, O., BENNACEUR-GRISCELLI, A.,
880 TACHDJIAN, G., PESCHANSKI, M. & PERRIER, A. L. 2008. Human embryonic stem cells
881 reveal recurrent genomic instability at 20q11.21. *Nat Biotechnol,* 26**,** 1364-6.
882 LIU, J. C., GUAN, X., RYAN, J. A., RIVERA, A. G., MOCK, C., AGRAWAL, V., AGARWAL,
883 V., LETAI, A., LEROU, P. H. & LAHAV, G. 2013. High mitochondrial priming sensitizes
884 hESCs to DNA-damage-induced apoptosis. *Cell Stem Cell,* 13**,** 483-91.
885 LUKAS, C., SAVIC, V., BEKKER-JENSEN, S., DOIL, C., NEUMANN, B., PEDERSEN, R. S.,
886 GRØFTE, M., CHAN, K. L., HICKSON, I. D., BARTEK, J. & LUKAS, J. 2011. 53BP1
887 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes
888 under replication stress. *Nat Cell Biol,* 13**,** 243-53.
889 LUO, L. Z., GOPALAKRISHNA-PILLAI, S., NAY, S. L., PARK, S. W., BATES, S. E., ZENG, X.,
890 IVERSON, L. E. & O'CONNOR, T. R. 2012. DNA repair in human pluripotent stem cells is
891 distinct from that in non-pluripotent human cells. *PLoS One,* 7**,** e30541.
892 MA, X., ZHENG, W., WEI, D., MA, Y., WANG, T., WANG, J., LIU, Q. & YANG, S. 2006. High-
893 level expression, purification and pro-apoptosis activity of HIV-TAT-survivin (T34A) mutant
894 to cancer cells in vitro. *J Biotechnol,* 123**,** 367-78.
895 MADDEN, D. T., DAVILA-KRUGER, D., MELOV, S. & BREDESEN, D. E. 2011. Human
896 embryonic stem cells express elevated levels of multiple pro-apoptotic BCL-2 family
897 members. *PLoS One,* 6**,** e28530.
898 MALKIN, D., LI, F. P., STRONG, L. C., FRAUMENI, J. F., NELSON, C. E., KIM, D. H., KASSEL,
899 J., GRYKA, M. A., BISCHOFF, F. Z. & TAINSKY, M. A. 1990. Germ line p53 mutations
900 in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science,* 250**,** 1233-
901 8.
902 MANDAI, M., KURIMOTO, Y. & TAKAHASHI, M. 2017. Autologous Induced Stem-Cell-Derived
903 Retinal Cells for Macular Degeneration. *N Engl J Med,* 377**,** 792-793.
904 MARKOULI, C., COUVREU DE DECKERSBERG, E., REGIN, M., NGUYEN, H. T.,
905 ZAMBELLI, F., KELLER, A., DZIEDZICKA, D., DE KOCK, J., TILLEMAN, L., VAN
906 NIEUWERBURGH, F., FRANCESCHINI, L., SERMON, K., GEENS, M. & SPITS, C.
907 2019. Gain of 20q11.21 in Human Pluripotent Stem Cells Impairs TGF-β-Dependent
908 Neuroectodermal Commitment. *Stem Cell Reports,* 13**,** 163-176.
909 MAYNARD, S., SWISTOWSKA, A. M., LEE, J. W., LIU, Y., LIU, S. T., DA CRUZ, A. B., RAO,
910 M., DE SOUZA-PINTO, N. C., ZENG, X. & BOHR, V. A. 2008. Human embryonic stem
911 cells have enhanced repair of multiple forms of DNA damage. *Stem Cells,* 26**,** 2266-74.
912 MERKLE, F. T., GHOSH, S., KAMITAKI, N., MITCHELL, J., AVIOR, Y., MELLO, C., KASHIN,
913 S., MEKHOUBAD, S., ILIC, D., CHARLTON, M., SAPHIER, G., HANDSAKER, R. E.,
914 GENOVESE, G., BAR, S., BENVENISTY, N., MCCARROLL, S. A. & EGGAN, K. 2017.
915 Human pluripotent stem cells recurrently acquire and expand dominant negative P53
916 mutations. *Nature,* 545**,** 229-233.
917 MESRI, M., WALL, N. R., LI, J., KIM, R. W. & ALTIERI, D. C. 2001. Cancer gene therapy using
918 a survivin mutant adenovirus. *J Clin Invest,* 108**,** 981-90.
919 MILHOLLAND, B., DONG, X., ZHANG, L., HAO, X., SUH, Y. & VIJG, J. 2017. Differences
920 between germline and somatic mutation rates in humans and mice. *Nat Commun,* 8**,** 15183.
921 MOMCILOVIĆ, O., CHOI, S., VARUM, S., BAKKENIST, C., SCHATTEN, G. & NAVARA, C.
922 2009. Ionizing radiation induces ataxia telangiectasia mutated-dependent checkpoint
923 signaling and G(2) but not G(1) cell cycle arrest in pluripotent human embryonic stem cells.
924 *Stem Cells,* 27**,** 1822-35.
925 MOSTOFI, F. K. & PRICE, E. B. 1973. Tumors of the male genital system. A.F.I.P. Atlas of Tumor

Pathology. 2nd series, Fascicle 8, Washington D.C.
NAIM, V., WILHELM, T., DEBATISSE, M. & ROSSELLI, F. 2013. ERCC1 and MUS81-EME1 promote sister chromatid separation by processing late replication intermediates at common fragile sites during mitosis. *Nat Cell Biol,* 15**,** 1008-15.
NGUYEN, H. T., GEENS, M. & SPITS, C. 2013. Genetic and epigenetic instability in human pluripotent stem cells. *Hum Reprod Update,* 19**,** 187-205.
NICHOLS, J. & SMITH, A. 2009. Naive and primed pluripotent states. *Cell Stem Cell,* 4**,** 487-92.
OHGUSHI, M., MATSUMURA, M., EIRAKU, M., MURAKAMI, K., ARAMAKI, T., NISHIYAMA, A., MUGURUMA, K., NAKANO, T., SUGA, H., UENO, M., ISHIZAKI, T., SUEMORI, H., NARUMIYA, S., NIWA, H. & SASAI, Y. 2010. Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. *Cell Stem Cell,* 7**,** 225-39.
OLARIU, V., HARRISON, N. J., COCA, D., GOKHALE, P. J., BAKER, D., BILLINGS, S., KADIRKAMANATHAN, V. & ANDREWS, P. W. 2010. Modeling the evolution of culture-adapted human embryonic stem cells. *Stem Cell Res,* 4**,** 50-6.
OOSTERHUIS, J. W., ANDREWS, P. W., KNOWLES, B. B. & DAMJANOV, I. 1984. Effects of cis-platinum on embryonal carcinoma cell lines in vitro. *Int J Cancer,* 34**,** 133-9.
PETLJAK, M., ALEXANDROV, L. B., BRAMMELD, J. S., PRICE, S., WEDGE, D. C., GROSSMANN, S., DAWSON, K. J., JU, Y. S., IORIO, F., TUBIO, J. M. C., KOH, C. C., GEORGAKOPOULOS-SOARES, I., RODRÍGUEZ-MARTÍN, B., OTLU, B., O'MEARA, S., BUTLER, A. P., MENZIES, A., BHOSLE, S. G., RAINE, K., JONES, D. R., TEAGUE, J. W., BEAL, K., LATIMER, C., O'NEILL, L., ZAMORA, J., ANDERSON, E., PATEL, N., MADDISON, M., NG, B. L., GRAHAM, J., GARNETT, M. J., MCDERMOTT, U., NIK-ZAINAL, S., CAMPBELL, P. J. & STRATTON, M. R. 2019. Characterizing Mutational Signatures in Human Cancer Cell Lines Reveals Episodic APOBEC Mutagenesis. *Cell,* 176**,** 1282-1294.e20.
PICKERING, M. T. & KOWALIK, T. F. 2006. Rb inactivation leads to E2F1-mediated DNA double-strand break accumulation. *Oncogene,* 25**,** 746-55.
PLANTAZ, D., MOHAPATRA, G., MATTHAY, K. K., PELLARIN, M., SEEGER, R. C. & FEUERSTEIN, B. G. 1997. Gain of chromosome 17 is the most frequent abnormality detected in neuroblastoma by comparative genomic hybridization. *Am J Pathol,* 150**,** 81-9.
PRICE, C. J., STAVISH, D., GOKHALE, P. J., SARGEANT, S., LACEY, J., RODRIGUEZ, T. A. & BARBARIC, I. 2019. Genetically variant human pluripotent stem cells selectively eliminate wild-type counterparts through YAP-mediated cell competition. *bioRxiv***,** 854430.
REUBINOFF, B. E., PERA, M. F., FONG, C. Y., TROUNSON, A. & BONGSO, A. 2000. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat Biotechnol,* 18**,** 399-404.
RODRIGUEZ, S., JAFER, O., GOKER, H., SUMMERSGILL, B. M., ZAFARANA, G., GILLIS, A. J., VAN GURP, R. J., OOSTERHUIS, J. W., LU, Y. J., HUDDART, R., COOPER, C. S., CLARK, J., LOOIJENGA, L. H. & SHIPLEY, J. M. 2003. Expression profile of genes from 12p in testicular germ cell tumors of adolescents and adults associated with i(12p) and amplification at 12p11.2-p12.1. *Oncogene,* 22**,** 1880-91.
ROUHANI, F. J., NIK-ZAINAL, S., WUSTER, A., LI, Y., CONTE, N., KOIKE-YUSA, H., KUMASAKA, N., VALLIER, L., YUSA, K. & BRADLEY, A. 2016. Mutational History of a Human Cell Lineage from Somatic to Induced Pluripotent Stem Cells. *PLoS Genet,* 12**,** e1005932.
SARETZKI, G., WALTER, T., ATKINSON, S., PASSOS, J. F., BARETH, B., KEITH, W. N., STEWART, R., HOARE, S., STOJKOVIC, M., ARMSTRONG, L., VON ZGLINICKI, T. & LAKO, M. 2008. Downregulation of multiple stress defense mechanisms during differentiation of human embryonic stem cells. *Stem Cells,* 26**,** 455-64.
SATO, Y., BANDO, H., DI PIAZZA, M., GOWING, G., HERBERTS, C., JACKMAN, S., LEONI, G., LIBERTINI, S., MACLACHLAN, T., MCBLANE, J. W., PEREIRA MOURIÈS, L.,

SHARPE, M., SHINGLETON, W., SURMACZ-CORDLE, B., YAMAMOTO, K. & VAN DER LAAN, J. W. 2019. Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider. *Cytotherapy,* 21**,** 1095-1111.

SCHWARTZ, S. D., HUBSCHMAN, J. P., HEILWELL, G., FRANCO-CARDENAS, V., PAN, C. K., OSTRICK, R. M., MICKUNAS, E., GAY, R., KLIMANSKAYA, I. & LANZA, R. 2012. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet,* 379**,** 713-20.

SIMARA, P., TESAROVA, L., REHAKOVA, D., MATULA, P., STEJSKAL, S., HAMPL, A. & KOUTNA, I. 2017. DNA double-strand breaks in human induced pluripotent stem cell reprogramming and long-term in vitro culturing. *Stem Cell Res Ther,* 8**,** 73.

SONG, W. K., PARK, K. M., KIM, H. J., LEE, J. H., CHOI, J., CHONG, S. Y., SHIM, S. H., DEL PRIORE, L. V. & LANZA, R. 2015. Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in Asian patients. *Stem Cell Reports,* 4**,** 860-72.

SPITS, C., MATEIZEL, I., GEENS, M., MERTZANIDOU, A., STAESSEN, C., VANDESKELDE, Y., VAN DER ELST, J., LIEBAERS, I. & SERMON, K. 2008. Recurrent chromosomal abnormalities in human embryonic stem cells. *Nat Biotechnol,* 26**,** 1361-3.

SRIVASTAVA, S., ZOU, Z. Q., PIROLLO, K., BLATTNER, W. & CHANG, E. H. 1990. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature,* 348**,** 747-9.

TAAPKEN, S. M., NISLER, B. S., NEWTON, M. A., SAMPSELL-BARRON, T. L., LEONHARD, K. A., MCINTIRE, E. M. & MONTGOMERY, K. D. 2011. Karotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. *Nat Biotechnol,* 29**,** 313-4.

TAKAHASHI, K., TANABE, K., OHNUKI, M., NARITA, M., ICHISAKA, T., TOMODA, K. & YAMANAKA, S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell,* 131**,** 861-72.

TECHNOLOGIES, S. 2018. *Challenges in Ensuring hPSC Quality. Stemcell.com* [Online]. Available: https://www.stemcell.com/nature-research-roundtable-hPSC-quality [Accessed].

THOMPSON, O., VON MEYENN, F., HEWITT, Z., ALEXANDER, J., WOOD, A., WEIGHTMAN, R., GREGORY, S., KRUEGER, F., ANDREWS, S., BARBARIC, I., GOKHALE, P. J., MOORE, H. D., REIK, W., MILO, M., NIK-ZAINAL, S., YUSA, K. & ANDREWS, P. W. 2020. Low rates of mutation in clinical grade human pluripotent stem cells under different culture conditions. *Nature Communications,* 11**,** 1528.

THOMSON, J. A., ITSKOVITZ-ELDOR, J., SHAPIRO, S. S., WAKNITZ, M. A., SWIERGIEL, J. J., MARSHALL, V. S. & JONES, J. M. 1998. Embryonic stem cell lines derived from human blastocysts. *Science,* 282**,** 1145-7.

TORRES, E. M., WILLIAMS, B. R. & AMON, A. 2008. Aneuploidy: cells losing their balance. *Genetics,* 179**,** 737-46.

VALLABHANENI, H., LYNCH, P. J., CHEN, G., PARK, K., LIU, Y., GOEHE, R., MALLON, B. S., BOEHM, M. & HURSH, D. A. 2018. High Basal Levels of γH2AX in Human Induced Pluripotent Stem Cells Are Linked to Replication-Associated DNA Damage and Repair. *Stem Cells,* 36**,** 1501-1513.

VAN ECHTEN-ARENDS, J., MASTENBROEK, S., SIKKEMA-RADDATZ, B., KOREVAAR, J. C., HEINEMAN, M. J., VAN DER VEEN, F. & REPPING, S. 2011. Chromosomal mosaicism in human preimplantation embryos: a systematic review. *Hum Reprod Update,* 17**,** 620-7.

VIEL, A., BRUSELLES, A., MECCIA, E., FORNASARIG, M., QUAIA, M., CANZONIERI, V., POLICICCHIO, E., URSO, E. D., AGOSTINI, M., GENUARDI, M., LUCCI-CORDISCO, E., VENESIO, T., MARTAYAN, A., DIODORO, M. G., SANCHEZ-METE, L., STIGLIANO, V., MAZZEI, F., GRASSO, F., GIULIANI, A., BAIOCCHI, M., MAESTRO, R., GIANNINI, G., TARTAGLIA, M., ALEXANDROV, L. B. & BIGNAMI, M. 2017. A

Specific Mutational Signature Associated with DNA 8-Oxoguanine Persistence in MUTYH-defective Colorectal Cancer. *EBioMedicine,* 20**,** 39-49.

WATANABE, K., UENO, M., KAMIYA, D., NISHIYAMA, A., MATSUMURA, M., WATAYA, T., TAKAHASHI, J. B., NISHIKAWA, S., MUGURUMA, K. & SASAI, Y. 2007. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat Biotechnol,* 25**,** 681-6.

WERBOWETSKI-OGILVIE, T. E., BOSSÉ, M., STEWART, M., SCHNERCH, A., RAMOS-MEJIA, V., ROULEAU, A., WYNDER, T., SMITH, M. J., DINGWALL, S., CARTER, T., WILLIAMS, C., HARRIS, C., DOLLING, J., WYNDER, C., BOREHAM, D. & BHATIA, M. 2009. Characterization of human embryonic stem cells with features of neoplastic progression. *Nat Biotechnol,* 27**,** 91-7.

YANG, D., WELM, A. & BISHOP, J. M. 2004. Cell division and cell survival in the absence of survivin. *Proc Natl Acad Sci U S A,* 101**,** 15100-5.

YASUDA, S., KUSAKAWA, S., KURODA, T., MIURA, T., TANO, K., TAKADA, N., MATSUYAMA, S., MATSUYAMA, A., NASU, M., UMEZAWA, A., HAYAKAWA, T., TSUTSUMI, H. & SATO, Y. 2018. Tumorigenicity-associated characteristics of human iPS cell lines. *PLoS One,* 13**,** e0205022.

YU, J., VODYANIK, M. A., SMUGA-OTTO, K., ANTOSIEWICZ-BOURGET, J., FRANE, J. L., TIAN, S., NIE, J., JONSDOTTIR, G. A., RUOTTI, V., STEWART, R., SLUKVIN, I. I. & THOMSON, J. A. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science,* 318**,** 1917-20.

ZHANG, J., HIRST, A. J., DUAN, F., QIU, H., HUANG, R., JI, Y., BAI, L., ZHANG, F., ROBINSON, D., JONES, M., LI, L., WANG, P., JIANG, P., ANDREWS, P. W., BARBARIC, I. & NA, J. 2019. Anti-apoptotic Mutations Desensitize Human Pluripotent Stem Cells to Mitotic Stress and Enable Aneuploid Cell Survival. *Stem Cell Reports,* 12**,** 557-571.

FIGURE LEGENDS

Figure 1: Appearance of mutations in human PSC involves both mutation and selection.
Human PSC are subject to the full range of mutations seen in other systems, including single base changes and small insertions and deletions (INDELS) (Thompson et al., 2020), as well as larger scale genomic rearrangements with gains or losses of whole chromosomes or chromosomal fragments that alter the number of copies of whole sets of genes, and consequently their levels of expression (Enver et al., 2005). The most commonly seen chromosomal rearrangements are illustrated. Generally, in the absence of a clonogenic bottleneck, these mutations will never be detected unless they offer the mutant cell a selective growth advantage (Olariu et al., 2010).

Figure 2: Nature of tumors derived from pluripotent stem cells
PSC produce tumours that may contain both undifferentiated PSC together with their differentiated derivatives, in which case the tumor is termed a 'Teratocarcinoma' and is regarded as highly malignant. If the PSC fully differentiate, so that the tumor contains only differentiated derivatives, it is termed a 'Teratoma' and is generally regarded as benign and not malignant (Damjanov and Andrews, 2007a, Damjanov and Andrews, 2016). However, a caveat is that some of the differentiated derivatives may develop into a secondary malignancy corresponding to their particular cell type, and this may be driven by a mutation present in the parent PSC. In addition to somatic derivatives, PSC may also generate primitive endoderm elements which are precursors of highly malignant yolk sac carcinomas

Figure 3: Mechanisms of Variant Growth Advantage
A variety of mechanisms can be envisaged by which variant cells could gain a growth advantage over wild type cells. **Cell autonomous mechanisms:** mutation **(a)** drives faster cell cycle, or **(b)** blocks differentiation, or **(c)** blocks apoptosis. **Cell interactive mechanisms**: mutation **(d)** causes variant cell to inhibit the growth of its wild-type counterpart (Price et al., 2019) or **(e)** alters the patterns of differentiation. The latter could generate a selective advantage either if certain differentiated derivatives produced factors that promote differentiation (advantage would derive from blocking such lineages) or produced factors that blocked general differentiation (advantage would derive from enhancing differentiation to such lineages).

Figure 4: Overview of the origins of mutation in human PSC. a) Somatic cellular proliferation occurs predominantly without replication stress, allowing for faithful cell division. However, should replication stress occur, somatic cells can respond with cellular senescence, apoptosis or DNA repair. **b)** Human pluripotent stem cells proliferate rapidly and are susceptible to replication stress, DNA damage and mitotic errors. However, they display a low mutation rate, which is reconciled by responding to genetic stresses with apoptosis and efficient DNA repair. **c)** Culture of human PSC with exogenous nucleoside alleviates replication stress, DNA damage and mitotic errors. Alleviating genetic stress minimises apoptosis, enhancing growth rate and removing the selective advantage of anti-apoptotic mutations.

BOX 1. Detection of Genetically Variant Cells

Routine monitoring of PSC cultures for the appearance of genetic variants is essential: the proportion of some common variants in a culture can rise from undetectable to 100% within 5 -10 passages (Olariu et al., 2010). Different screening methods have different advantages and limitations, including the minimum proportions of variant cells that can be detected in mosaic populations, and whether prior knowledge of the variant to be detected is required. Other factors to consider are the types of variant detectable, cost, speed and whether specialist facilities and expertise are required. At a minimum we would recommend regular screening of cultures for common variants using the PCR method, which is rapid and can easily be carried out in most laboratories, backed up at key decision points in specific experiments with more in-depth analysis using other methods as appropriate.

- **Karyotyping by G banding:** Indiscriminate view of structural and numerical chromosomal abnormalities greater than 5Mb (Steinemann et al., 2013). Limited to analysis of proliferating cells, but the only convenient method to detect balanced genomic rearrangements. Sensitivity and precision can be enhanced by use of fluorescent hybridization probes. Minimum mosaicism detected: 18% (routine analysis of 30 metaphase spreads), 6% (analysis of 100 metaphase spreads) (Baker et al., 2016).
- **Array Comparative Genome Hybridisation (aCGH) and SNP array:** Global screening detects copy number variants down to 1Kb. Minimum mosaicism detected: 10-15% for small copy number variants. (Valli et al., 2011).
- **Interphase Fluorescent In Situ Hybridisation (FISH):** Hybridisation of fluorescent probes to highly complementary nucleic acid sequences in interphase cells. Requires prior knowledge of the copy number variant to be detected. The presence of false negatives dependent upon the type of variant restricts the sensitivity of detection: Minimum mosaicism detected depends upon the number of nuclei assessed and the size of the CNV: ~5% is possible for gains of whole chromosome arms, with 100 nuclei assessed, but this falls markedly for smaller CNV (Baker et al., 2016).
- **qPCR based assays:** Rapid method suitable for any well-equipped molecular cell biology laboratories. Requires prior knowledge of the CNV to be detected. Minimum mosaicism detected: ~5% (Baker et al., 2016).
- **Expression karyotyping:** Regions of CNV are detected based on their global gene expression from RNA-seq data in comparison to a diploid calibrator sample. Requires no prior knowledge of the CNV to be detected, although it is only capable of detecting chromosomal aberrations greater than ~10Mb with a sensitivity of ~30% (Mayshar et al., 2010).

- **eSNP Karyotyping:** An adaptation of expression karyotyping with allele bias. This approach does not require a diploid calibrator sample for CNV detection. Resolution of eSNP karyotyping depends on the genomic region and sequencing depth, yet it comfortably detected amplifications to entire chromosomes or chromosome arms. The eSNP karyotyping approach shows comparable sensitivity to other DNA based array approaches (~30%) (Weissbein et al., 2016).

BAKER, D., HIRST, A. J., GOKHALE, P. J., JUAREZ, M. A., WILLIAMS, S., WHEELER, M., BEAN, K., ALLISON, T. F., MOORE, H. D., ANDREWS, P. W. & BARBARIC, I. 2016. Detecting Genetic Mosaicism in Cultures of Human Pluripotent Stem Cells. *Stem Cell Reports,* 7**,** 998-1012.

MAYSHAR, Y., BEN-DAVID, U., LAVON, N., BIANCOTTI, J. C., YAKIR, B., CLARK, A. T., PLATH, K., LOWRY, W. E. & BENVENISTY, N. 2010. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell,* 7**,** 521-31.

OLARIU, V., HARRISON, N. J., COCA, D., GOKHALE, P. J., BAKER, D., BILLINGS, S., KADIRKAMANATHAN, V. & ANDREWS, P. W. 2010. Modeling the evolution of culture-adapted human embryonic stem cells. *Stem Cell Res,* 4**,** 50-6.

STEINEMANN, D., GÖHRING, G. & SCHLEGELBERGER, B. 2013. Genetic instability of modified stem cells - a first step towards malignant transformation? *Am J Stem Cells,* 2**,** 39-51.

VALLI, R., MARLETTA, C., PRESSATO, B., MONTALBANO, G., LO CURTO, F., PASQUALI, F. & MASERATI, E. 2011. Comparative genomic hybridization on microarray (a-CGH) in constitutional and acquired mosaicism may detect as low as 8% abnormal cells. *Mol Cytogenet,* 4**,** 13.

WEISSBEIN, U., SCHACHTER, M., EGLI, D. & BENVENISTY, N. 2016. Analysis of chromosomal aberrations and recombination by allelic bias in RNA-Seq. *Nat Commun,* 7**,** 12144.

BOX 2: Features of common genetic changes in PSC.

Gains:

Chromosome 1q

- Typically acquired as a structural chromosome rearrangement (unbalanced translocation or interstitial duplication) (Amps et al., 2011, Baker et al., 2016)
- Minimal region: 1q25-q41 (Baker et al., 2016)
- Potential driver gene: *MDM4*
- Association with cancer: Wilms' tumor (Chagtai et al., 2016), multiple myeloma (Walker et al., 2010) and intracranial ependymomas (Kilday et al., 2012)

Chromosome 12p

- Typically acquired as a whole chromosome trisomy, although a number of studies have also reported a partial trisomy of the short arm of chromosome 12 (Amps et al., 2011, Baker et al., 2016)
- Minimal region: 12p11-pter (Baker et al., 2016)
- Potential driver gene: *NANOG*
- Functional consequences observed to date: increased proliferation rate due to increased replication, reduced propensity for spontaneous differentiation and apoptosis (Ben-David et al., 2014)
- Association with cancer: testicular germ cell tumors (Atkin and Baker, 1982)

Chromosome 17q

- Typically acquired as a structural chromosome rearrangement (unbalanced translocation or interstitial duplication) (Amps et al., 2011, Baker et al., 2016)
- Minimal region: 17q25-qter (Baker et al., 2016)
- Potential driver gene: *SURVIVIN* (*BIRC5)*
- Functional consequences observed to date: growth advantage in undifferentiated cultures (Olariu et al., 2010) due to enhanced proliferation (Lee et al., 2015); faster differentiation to mesodiencephalic dopaminergic neural cells compared with wild-type cells (Lee et al., 2015)
- Association with cancer: testicular germ cell tumors (Kraggerud et al., 2002) and neuroblastoma (Bown et al., 2001)

Chromosome 20q

- Typically acquired as an interstitial duplication (Amps et al., 2011)
- Minimal region: 20q11.21 (Amps et al., 2011)
- Driver gene: *BCL2L1* (Avery et al., 2013, Nguyen et al., 2013)
- Functional consequences observed to date: growth advantage due to reduced propensity for apoptosis (Avery et al., 2013, Nguyen et al., 2013), reduced dependence on bFGF (Werbowetski-Ogilvie et al., 2009), delayed differentiation to neural lineages (Werbowetski-Ogilvie et al., 2009) and reduced efficiency of neuroectodermal lineage commitment (Markouli et al., 2019)
- Association with cancer: colorectal cancer (Nguyen and Duong, 2018)

Losses:

Chromosome 10p

- Typically acquired as an interstitial deletion (Amps et al., 2011)
- Minimal region: 10p13-pter (Amps et al., 2011, Baker et al., 2016)
- Association with cancer: melanoma (Robertson et al., 1999) and glioblastoma (Kimmelman et al., 1996)

Chromosome 18q

- Typically acquired as an interstitial deletion (Amps et al., 2011)
- Minimal region: 18q21-qter (Amps et al., 2011, Baker et al., 2016)
- Association with cancer: colorectal carcinoma (Popat and Houlston, 2005)

AMPS, K., ANDREWS, P. W., ANYFANTIS, G., ARMSTRONG, L., AVERY, S., BAHARVAND, H., BAKER, J., BAKER, D., MUNOZ, M. B., BEIL, S., BENVENISTY, N., BEN-YOSEF, D., BIANCOTTI, J. C., BOSMAN, A., BRENA, R. M., BRISON, D., CAISANDER, G., CAMARASA, M. V., CHEN, J., CHIAO, E., CHOI, Y. M., CHOO, A. B., COLLINS, D., COLMAN, A., CROOK, J. M., DALEY, G. Q., DALTON, A., DE SOUSA, P. A., DENNING, C., DOWNIE, J., DVORAK, P., MONTGOMERY, K. D., FEKI, A., FORD, A., FOX, V., FRAGA, A. M., FRUMKIN, T., GE, L., GOKHALE, P. J., GOLAN-LEV, T., GOURABI, H., GROPP, M., LU, G., HAMPL, A., HARRON, K., HEALY, L., HERATH, W., HOLM, F., HOVATTA, O., HYLLNER, J., INAMDAR, M. S., IRWANTO, A. K., ISHII, T., JACONI, M., JIN, Y., KIMBER, S., KISELEV, S., KNOWLES, B. B., KOPPER, O., KUKHARENKO, V., KULIEV, A., LAGARKOVA, M. A., LAIRD, P. W., LAKO, M., LASLETT, A. L., LAVON, N., LEE, D. R., LEE, J. E., LI, C., LIM, L. S., LUDWIG, T. E., MA, Y., MALTBY, E., MATEIZEL, I., MAYSHAR, Y., MILEIKOVSKY, M., MINGER, S. L., MIYAZAKI, T., MOON, S. Y., MOORE, H., MUMMERY, C., NAGY, A., NAKATSUJI, N., NARWANI, K., OH, S. K., OLSON, C., OTONKOSKI, T., PAN, F., PARK, I. H., PELLS, S., PERA, M. F., PEREIRA, L. V., QI, O., RAJ, G. S., REUBINOFF, B., ROBINS, A., ROBSON, P., ROSSANT, J., SALEKDEH, G. H., SCHULZ, T. C., et al. 2011. Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. *Nat Biotechnol,* 29**,** 1132-44.

ATKIN, N. B. & BAKER, M. C. 1982. Specific chromosome change, i(12p), in testicular tumours? *Lancet,* 2**,** 1349.

AVERY, S., HIRST, A. J., BAKER, D., LIM, C. Y., ALAGARATNAM, S., SKOTHEIM, R. I., LOTHE, R. A., PERA, M. F., COLMAN, A., ROBSON, P., ANDREWS, P. W. & KNOWLES, B. B. 2013. BCL-XL mediates the strong selective advantage of a 20q11.21 amplification commonly found in human embryonic stem cell cultures. *Stem Cell Reports,* 1**,** 379-86.

BAKER, D., HIRST, A. J., GOKHALE, P. J., JUAREZ, M. A., WILLIAMS, S., WHEELER, M., BEAN, K., ALLISON, T. F., MOORE, H. D., ANDREWS, P. W. & BARBARIC, I. 2016. Detecting Genetic Mosaicism in Cultures of Human Pluripotent Stem Cells. *Stem Cell Reports,* 7**,** 998-1012.

BEN-DAVID, U., ARAD, G., WEISSBEIN, U., MANDEFRO, B., MAIMON, A., GOLAN-LEV, T., NARWANI, K., CLARK, A. T., ANDREWS, P. W., BENVENISTY, N. & CARLOS BIANCOTTI, J. 2014. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat Commun,* 5**,** 4825.

BOWN, N., LASTOWSKA, M., COTTERILL, S., O'NEILL, S., ELLERSHAW, C., ROBERTS, P., LEWIS, I., PEARSON, A. D. & GROUP, U. K. C. C. G. A. T. U. K. C. S. C. S. 2001. 17q gain in neuroblastoma predicts adverse clinical outcome. U.K. Cancer Cytogenetics Group and the U.K. Children's Cancer Study Group. *Med Pediatr Oncol,* 36**,** 14-9.

CHAGTAI, T., ZILL, C., DAINESE, L., WEGERT, J., SAVOLA, S., POPOV, S., MIFSUD, W., VUJANIĆ, G., SEBIRE, N., LE BOUC, Y., AMBROS, P. F., KAGER, L., O'SULLIVAN, M. J., BLAISE, A., BERGERON, C., MENGELBIER, L. H., GISSELSSON, D., KOOL, M., TYTGAT, G. A., VAN DEN HEUVEL-EIBRINK, M. M., GRAF, N., VAN TINTEREN, H., COULOMB, A., GESSLER, M., WILLIAMS, R. D. & PRITCHARD-JONES, K. 2016. Gain of 1q As a Prognostic Biomarker in Wilms Tumors (WTs) Treated

With Preoperative Chemotherapy in the International Society of Paediatric Oncology (SIOP) WT 2001 Trial: A SIOP Renal Tumours Biology Consortium Study. *J Clin Oncol,* 34**,** 3195-203.

KILDAY, J. P., MITRA, B., DOMERG, C., WARD, J., ANDREIUOLO, F., OSTESO-IBANEZ, T., MAUGUEN, A., VARLET, P., LE DELEY, M. C., LOWE, J., ELLISON, D. W., GILBERTSON, R. J., COYLE, B., GRILL, J. & GRUNDY, R. G. 2012. Copy number gain of 1q25 predicts poor progression-free survival for pediatric intracranial ependymomas and enables patient risk stratification: a prospective European clinical trial cohort analysis on behalf of the Children's Cancer Leukaemia Group (CCLG), Societe Francaise d'Oncologie Pediatrique (SFOP), and International Society for Pediatric Oncology (SIOP). *Clin Cancer Res,* 18**,** 2001-11.

KIMMELMAN, A. C., ROSS, D. A. & LIANG, B. C. 1996. Loss of heterozygosity of chromosome 10p in human gliomas. *Genomics,* 34**,** 250-4.

KRAGGERUD, S. M., SKOTHEIM, R. I., SZYMANSKA, J., EKNAES, M., FOSSÅ, S. D., STENWIG, A. E., PELTOMÄKI, P. & LOTHE, R. A. 2002. Genome profiles of familial/bilateral and sporadic testicular germ cell tumors. *Genes Chromosomes Cancer,* 34**,** 168-74.

LEE, C. T., BENDRIEM, R. M., KINDBERG, A. A., WORDEN, L. T., WILLIAMS, M. P., DRGON, T., MALLON, B. S., HARVEY, B. K., RICHIE, C. T., HAMILTON, R. S., CHEN, J., ERRICO, S. L., TSAI, S. Y., UHL, G. R. & FREED, W. J. 2015. Functional consequences of 17q21.31/WNT3-WNT9B amplification in hPSCs with respect to neural differentiation. *Cell Rep,* 10**,** 616-32.

MARKOULI, C., COUVREU DE DECKERSBERG, E., REGIN, M., NGUYEN, H. T., ZAMBELLI, F., KELLER, A., DZIEDZICKA, D., DE KOCK, J., TILLEMAN, L., VAN NIEUWERBURGH, F., FRANCESCHINI, L., SERMON, K., GEENS, M. & SPITS, C. 2019. Gain of 20q11.21 in Human Pluripotent Stem Cells Impairs TGF-β-Dependent Neuroectodermal Commitment. *Stem Cell Reports,* 13**,** 163-176.

NGUYEN, H. T. & DUONG, H. Q. 2018. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. *Oncol Lett,* 16**,** 9-18.

NGUYEN, H. T., GEENS, M. & SPITS, C. 2013. Genetic and epigenetic instability in human pluripotent stem cells. *Hum Reprod Update,* 19**,** 187-205.

OLARIU, V., HARRISON, N. J., COCA, D., GOKHALE, P. J., BAKER, D., BILLINGS, S., KADIRKAMANATHAN, V. & ANDREWS, P. W. 2010. Modeling the evolution of culture-adapted human embryonic stem cells. *Stem Cell Res,* 4**,** 50-6.

POPAT, S. & HOULSTON, R. S. 2005. A systematic review and meta-analysis of the relationship between chromosome 18q genotype, DCC status and colorectal cancer prognosis. *Eur J Cancer,* 41**,** 2060-70.

ROBERTSON, G. P., HERBST, R. A., NAGANE, M., HUANG, H. J. & CAVENEE, W. K. 1999. The chromosome 10 monosomy common in human melanomas results from loss of two separate tumor suppressor loci. *Cancer Res,* 59**,** 3596-601.

WALKER, B. A., LEONE, P. E., CHIECCHIO, L., DICKENS, N. J., JENNER, M. W., BOYD, K. D., JOHNSON, D. C., GONZALEZ, D., DAGRADA, G. P., PROTHEROE, R. K., KONN, Z. J., STOCKLEY, D. M., GREGORY, W. M., DAVIES, F. E., ROSS, F. M. & MORGAN, G. J. 2010. A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. *Blood,* 116**,** e56-65.

WERBOWETSKI-OGILVIE, T. E., BOSSÉ, M., STEWART, M., SCHNERCH, A., RAMOS-MEJIA, V., ROULEAU, A., WYNDER, T., SMITH, M. J., DINGWALL, S., CARTER, T., WILLIAMS, C., HARRIS, C., DOLLING, J., WYNDER, C., BOREHAM, D. & BHATIA, M. 2009. Characterization of human embryonic stem cells with features of neoplastic progression. *Nat Biotechnol,* 27**,** 91-7.