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Review

Genomic instability, driver genes and cell selection: Projections from cancer to stem cells ☆

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ABSTRACT

Cancer cells and stem cells share many traits, including a tendency towards genomic instability. Human cancers exhibit tumor-specific genomic aberrations, which often affect their malignancy and drug response. During their culture propagation, human pluripotent stem cells (hPSCs) also acquire characteristic genomic aberrations, which may have significant impact on their molecular and cellular phenotypes. These aberrations vary in size from single nucleotide alterations to copy number alterations to whole chromosome gains. A prominent challenge in both cancer and stem cell research is to identify "driver aberrations" that confer a selection advantage, and "driver genes" that underlie the recurrence of these aberrations. Following principles that are already well-established in cancer research, candidate driver genes have also been suggested in hPSCs. Experimental validation of the functional role of such candidates can uncover whether these are bona fide driver genes. The identification of driver genes may bring us closer to a mechanistic understanding of the genomic instability of stem cells. Guided by terminologies and methodologies commonly applied in cancer research, such understanding may have important ramifications for both stem cell and cancer biology. This article is part of a Special Issue entitled: Stress as a fundamental theme in cell plasticity.

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1. Introduction

1.1. Human pluripotent stem cells (hPSCs) resemble cancer cells

Human pluripotent stem cells (hPSCs) can be derived by isolating the inner cell mass of embryos at the blastocyst stage [1], by transient induction of transcription factors in somatic cells [2], or through somatic cell nuclear transfer [3]. Regardless of their cellular origin, however, hPSCs are fundamentally different from their in-vivo counterparts: while maintaining the ability to differentiate into any cell type of the human body, hPSCs acquire in culture an extraordinary self-renewal and proliferation capacities, which do not exist at all in the pluripotent cells of the blastocyst.

Indeed, the only cells that exhibit a parallel proliferative capacity are cancer cells, and the relationship between these cell types has therefore been at the focus of extensive research (reviewed in [4]). In line with the notion that induced pluripotency and tumorigenic transformation are related processes [5], hPSCs share many hallmark characteristics with human cancer cells, including similar genomic [6,7] and epigenomic [8,9] landscapes, unique activation of some signal transduction pathways (e.g., [10]), high levels of telomerase activity [11] and lack of contact inhibition [1]. Undoubtedly, one of the most striking similarities between hPSCs and human cancer cells is their genomic instability [12]; both hPSCs and cancer cells are genomically unstable, and are prone to acquire genetic aberrations at the levels of whole chromosomes, sub-chromosomal loci or single genes (reviewed in [13–15]).

1.2. Genomic instability in cancer cells and in hPSCs

One of the most dramatic manifestations of cellular adaptation to a stressful environment is the stable acquisition of genomic aberrations. These alterations in the genomic material can range in size from a single base to an entire chromosome, and can consequently affect the expression of a single gene (in the case of a point mutation), of several genes (in the case of small gains and deletions), or even of thousands of genes simultaneously (in the case of aneuploidy). Unlike epigenetic changes, genetic changes are often irreversible, at least at the level of the single cell.

Both in cultures of hPSCs and in tumors, genomic aberrations initially lead to genomic variation, namely to a genetically-heterogeneous cell population [16–18]. However, under conditions that promote clonal selection, cells that harbor advantageous aberrations will soon outcompete their counterparts and thus give rise to the dominant, and often sole, surviving clone. Consistent with that, both human tumors and hPSC cultures eventually become clonal, and genomic aberrations that confer a selection advantage thus get fixed in the cell population [16,17,19].

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While cells in culture may be inherently unstable, cancer cells and pluripotent cells proliferate much faster than normal mature cell types, and — importantly — they do not senesce in culture. Therefore, these cell types are more prone to acquire genomic abnormalities, and these abnormalities may significantly increase their in vivo tumorigenicity [4]. Other factors that may make these cell types particularly vulnerable to genomic insults include unique cell cycle characteristics and DNA damage repair mechanisms, as well as cell culture practices and culture conditions (reviewed in [15]).

1.3. Uncovering driver genes that underlie genomic aberrations

Multiple studies have recently described in detail the recurrent genomic aberrations in hPSCs (reviewed in [13,15]). However, the causes and consequences of these aberrations remained largely unexplored until recently. Presently, it seems that the focus of the field is gradually shifting from descriptive studies that identify and characterize the aberrations to mechanistic studies that aim to unravel their potential “consequences” on the one hand, and their “causes” on the other hand.

A key challenge pertaining to the “cause” of genomic aberrations is the identification of underlying genes that drive recurrent aberrations. Adopting cancer research terminology, this problem could be phrased in terms of “driver” and “passenger” aberrations/mutations: which aberrations and which genes confer a substantial selection advantage, and how can these genes be distinguished from innocent bystanders? There are actually two interwoven challenges here: first, the driver aberration(s) need to be identified on the noisy background of other present genomic changes; second, the key genes that underlie the driver aberration(s) (i.e., the driver genes) have to be determined, often on the background of many other genes that reside within the aberrant region. As in cancer, the global genomic instability that characterizes late-stage cultures of hPSCs may mask both the driver aberrations and the driver genes, thus making their identification a rather difficult task.

2. Levels of genomic instability in hPSCs

As already mentioned, the genomic aberrations in hPSCs range in size from single nucleotide alterations (SNAs), to small gains and deletions (also referred to as copy number alterations, or CNAs), to trisomies or monosomies of whole chromosomes (that is, aneuploidy). This distinction is not merely technical, for different types of aberrations may stem from distinct defective mechanisms. This distinction may also be useful for the attempt to tease out the driver genes underlying the recurrent aberrations. The recurrent genomic aberrations in hPSCs, and the candidate driver genes that underlie these aberrations, are listed in Table 1.

2.1. Aneuploidy in hPSCs

Large chromosomal aberrations are commonly present in cultures of hPSCs. Whereas these aberrations are presumed to arise randomly, giving rise to karyotypically heterogeneous cultures [18], only few of them prevail and eventually take over the culture. Over a third of the human embryonic stem cell cultures exhibit identical chromosomal aberrations in over 5% of the cells [20]; and more than 10% of the hPSC cultures harbor at least one large chromosomal aberration in the majority of the cell population [12,21,22].

There are several lines of evidence that argue for the positive selection involved in the accumulation of large chromosomal aberrations in hPSC cultures. First, some chromosomal aberrations are significantly more common than others: trisomies of chromosomes 1, 12, 17 and X (or gains of one of their arms) are much more prevalent in hPSC cultures than any other trisomy or monosomy [12,19–24]. Second, chromosomal aberrations often take over the culture very rapidly, so that very few passages can go by from their first detection in rare cells to their widespread existence in culture [19,21]. Third, it has been recently shown that the most common aberration in hPSCs, trisomy 12, induces profound changes in the global gene expression signature of hPSCs, increasing their proliferation rate and their tumorigenicity [25]. Fourth, the same chromosomal aberrations that arise in hPSC cultures also arise in germ cell tumors in-vivo [12,19]. Fifth, some of the recurrent chromosomal aberrations in hPSCs are also observed in the syntenic chromosomes of mouse and monkey PSCs, suggesting their evolutionary-conservation [26]. Lastly, the chromosomal aberrations that arise in hPSC cultures are strikingly different from those that arise in embryogenesis [27], highlighting the role of the stressful environment in supporting the specific aberrations observed in culture.

Considering that only few aneuploidies facilitate the survival of hPSCs and allow them to prevail, a key question for each of these common aberrations is which gene(s) endow the cells with a selection advantage. As hPSCs acquire mainly trisomies, one would expect the driver genes to be over-expressed in the aneuploid cells. However, as large chromosomal aberrations encompass hundreds — and even thousands — of genes, it is difficult to determine which ones are the driver genes. It has been shown that 20%–40% of the genes within an aberrant region are significantly differentially expressed [21]; pinpointing one or few genes that are not only over-expressed in a trisomy, but are also essential for its accumulation, is therefore highly challenging. Nonetheless, as will be described below, application of several approaches has

Table 1
A list of recurrent genomic aberrations in hPSCs, and the candidate driver genes underlying these aberrations.

Key publication(s)	Supportive evidence or validation(s)	Proposed selection advantage	Candidate gene(s)	Recurrent genomic aberration
[20,21,29,52]	Consistent over-expression in aberrant cells Over-expression of *NANOG* jeopardizes differentiation Known role in cancer	Promoting self-renewal	*NANOG* *NANOGP1* *GDF3*	Trisomy 12 / Gain of 12p / Gain of 12p13.31
[19,26,55,56]	Over-expressed in aberrant cells Evolutionarily-conserved aberration Essential for the viability of hPSCs and for tumor formation Known role in cancer	Inhibiting apoptosis	*BIRC5* (*Survivin*)	Trisomy 17 / Gain of 17q
[26]	Over-expressed in aberrant cells Evolutionarily-conserved aberration Known role in cancer	Unknown	*ICT1*	
[20,31,32]	Over-expressed in aberrant cells Known role in cancer Direct evidence from knockdown and over-expression experiments	Inhibiting apoptosis	*BCL2L1* (*BCL-XL* isoform)	Gain of 20q11.21
[19]	–	Progressing cell cycle	Androgen receptor (*AR*)	Trisomy X

enabled the identification of several (candidate) driver genes underlying these large hPSC chromosomal aberrations.

2.2. Copy number alterations in hPSCs

Copy number alterations (CNAs), defined as deviations from the normal copy number of relatively small genomic loci, arise during the derivation and propagation of embryonic stem cells [28,29] and during the reprogramming of somatic cells into induced pluripotent stem cells [16,29]. Many of these CNAs may result from the normal variation that exists in the human population, or from rare genomic alterations that had already occurred prior to the derivation of the pluripotent cell lines. These alterations may have negative, neutral or positive adaptive value, and are thus expected to exhibit distinct dynamics: CNAs with negative adaptive value will disappear upon prolonged propagation in culture [16,28,29]; in contrast, CNAs with positive adaptive value will prevail [16,28,29]; due to the clonal nature of hPSC cultures, however, neutral CNAs may sometimes get fixed in the cell population as well [28,30], making it difficult to determine which CNAs really confer a selection advantage (that is, to distinguish the driver from the passenger CNAs).

In addition to the early-stage CNAs that arise during the derivation of hPSCs, other CNAs accumulate during long-term culture [16,28,29], in a similar manner to the chromosomal aberrations described in the previous section, albeit with distinct mechanisms underlying their creation [15]. Here again, specific CNAs are much more prevalent than others: amplification of 20q11.21 and 12p13.31 have been reported to occur in up to 25% and 13% of hPSC cultures, respectively [20,29]. As the minimal recurrent amplifications in these regions encompass only few genes, it is much easier to suggest candidate driver genes and prioritize them for experimental validation. Indeed, as will be described below, a candidate driver gene that resides within the 20q11.21 locus has been recently shown to mediate the strong selective advantage conferred by this amplification [31,32].

2.3. Point mutations in hPSCs

Point mutations, also referred to as single nucleotide alterations (SNAs), are a hallmark of human cancer. It is therefore not surprising that attempts have been made to identify driver mutations that facilitate the reprogramming process, or that provide culture-propagated hPSCs with a strong selection advantage. As somatic mutations exist in every human cell, and due to the clonality discussed above, the mere existence of a mutation in a hPSC line — even in most or all of the cells — is meaningless by itself. Applying massively-parallel sequencing techniques, multiple studies have tried to address this issue, and to identify recurrent SNAs that arise in hPSC cultures.

Surprisingly, perhaps, recurrent SNAs have not been identified to date in hPSCs [33–35]. Although these results ought to be considered with caution, given that only a few dozen of hPSC lines have been fully sequenced to date, no recurrent point mutation has been identified. Importantly, several mutations in cancer-related genes, such as genes of the p53 pathway, were reported to improve reprogramming efficiency [36,37]; however, none of the sequenced hPSC lines harbored naturally occurring mutations in this pathway, arguing against a selection advantage conferred by these mutations. Tackling this question from the opposite direction, a recent study manipulated the expression of genes that were found to be mutated in some hPSC lines, and found that none of these mutations facilitated reprogramming [35], further supporting the lack of an adaptive value to these mutations in hPSCs.

3. Identification of the driver genes underlying recurrent genomic aberrations

The similarity between the genomic instability of hPSCs and cancer cells suggests that the stem cell field may benefit from harnessing conceptual approaches and experimental technologies developed to uncover driving aberrations and genes in cancer. Not surprisingly, it seems that the same principles indeed guide the identification of functional aberrations and their underlying driver genes in both research arenas.

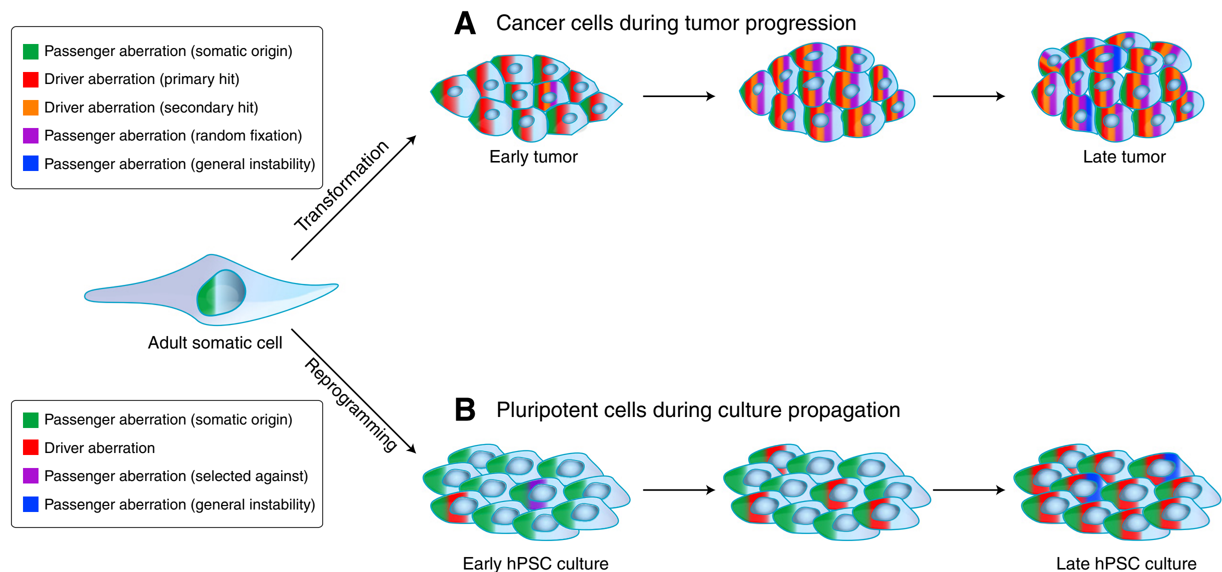
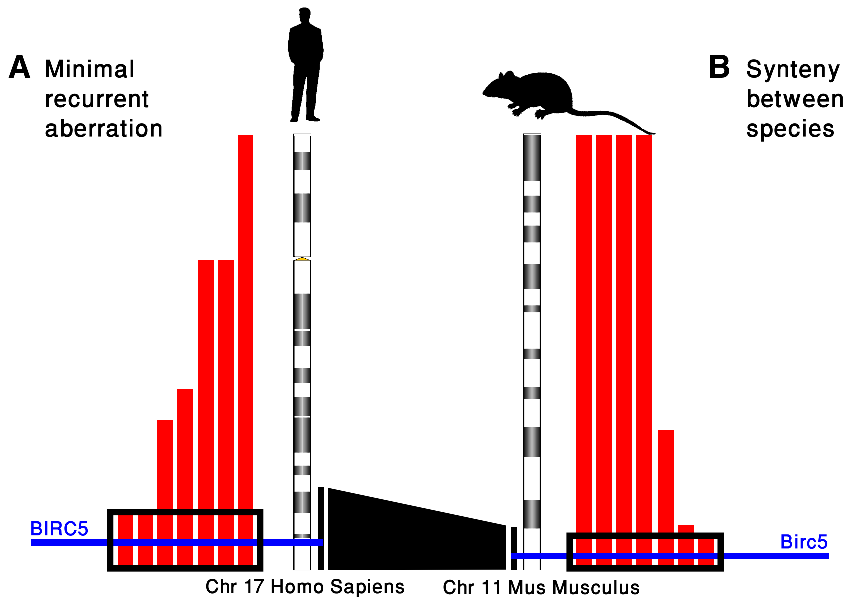
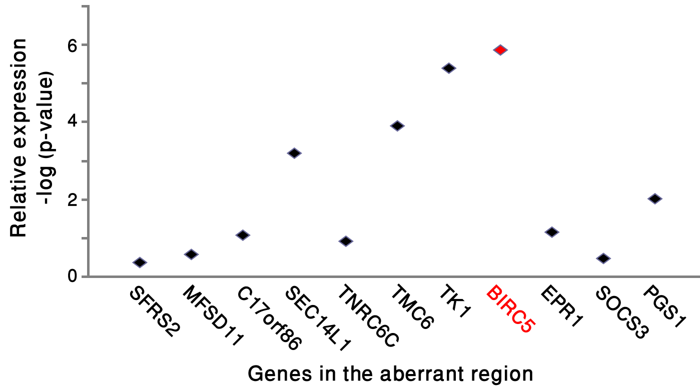
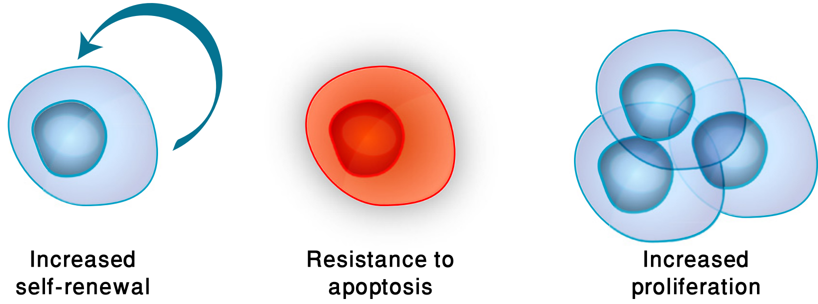
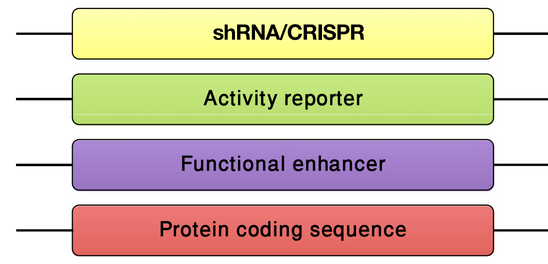


Fig. 1. Guidelines for the identification and prioritization of driver aberrations. (A) In cancer, driver aberrations progressively arise throughout tumor development, conferring various selection advantages that are required at each step of tumorigenesis. Passenger aberrations occur simultaneously, originating from the somatic tissue of origin, from random fixation (when occurring in the same cell as a driver aberration), or from general genomic instability at late stages of tumorigenesis. Aberrations are color-coded: red, orange and brown — driver aberrations; green, purple and blue — passenger aberrations. (B) In hPSCs, driver aberrations are expected to arise de novo throughout culture propagation, and accumulate in culture following further propagation. Aberrations are color-coded: green — aberrations originating from the cell of origin; purple — non-stable aberrations arising during the initial establishment of hPSC lines and disappearing following further propagation; red — stable aberrations arising during culture propagation, on a relatively diploid background; blue and brown — aberrations arising during the late stages of culture propagation, when genome-wide genomic instability is already evident. The red-coded aberrations are the best candidates to be "driver aberrations".


A Minimal recurrent aberration
B Synteny between species
BIRC5
Birc5
Chr 17 Homo Sapiens
Chr 11 Mus Musculus


C Differential expression
Relative expression -log (p-value)
0
2
4
6
SFRS2
MFSD11
C17orf86
SEC14L1
TNRC6C
TMC6
TK1
BIRC5
EPR1
SOCS3
PGS1
Genes in the aberrant region


D Functional role in relevant cellular processes
Increased self-renewal
Resistance to apoptosis
Increased proliferation


E Validation by genetic manipulation
shRNA/CRISPR
Activity reporter
Functional enhancer
Protein coding sequence

3.1. Identification and prioritization of candidate driver aberrations

3.1.1. High prevalence is an indicator of functionally important aberrations

The first rule of thumb in identifying meaningful genomic aberrations is that these aberrations are expected to be detected more often than other, non-meaningful ones. In line with this notion, all of the studies that addressed this issue — at the chromosomal, CNA or SNA levels — tried to identify statistically significant deviance from uniform or random distributions (for example [21,29,33]). By definition, positive adaptive value translates into higher survival rates; hence, if an aberration confers a strong selection advantage, it will most likely accumulate (and be detected) more often in the cell population.

Importantly, population-wide analyses are essential for identifying recurrent aberrations, due to the heterogeneous nature of hPSC cultures. Non-functional aberrations may exist in rare cells at any given time point [18,30], but only the functional, advantageous ones are expected to enable the cells that harbor them to survive, proliferate and take over the cell population. The recurrence of aberrations at the single cell level is therefore not necessarily indicative of their importance. For example, some aberrations may commonly arise in hPSCs simply due to their genomic location near a fragile site [16]; if there is a negative selection against these aberrations, however, they will soon disappear and will not get fixed in the population. Importantly, such aberrations may also be of neutral adaptive value, and in this case they may get fixed in the population by chance, leading to their common detection in the population despite not being true "driver" aberrations.

It is also important to note, that the recurrence of a specific aberration should not be considered a mandatory pre-requisite for the establishment of its importance. Perturbing a specific pathway may result in a selective advantage, which may be achieved through the alteration of various genes in this pathway, leading to various non-recurrent aberrations. Accordingly, gene ontology analyses of genes within identified CNAs (not all of which considered as "recurrent") revealed that these genes are associated with pluripotency pseudo-genes and with cancer-related genes [16,29]. Similarly, one study reported that point mutations in hPSCs were enriched for genes commonly mutated in cancer, although none of these mutations was recurrent by itself [33]. Therefore, high prevalence is a useful indication for potential functional importance, but it is neither necessary nor sufficient for the establishment of a driving role.

3.1.2. Driver aberrations are expected to arise de novo in early steps of culture propagation

In cancer, late-stage tumors often exhibit a complex karyotype, having acquired a genome-wide instability, often referred to as chromosomal instability (CIN) [38]. By that time, it may be extremely challenging to distinguish the driver mutations from the passenger mutations due to the noisy genomic background. On the other hand, the high copy number variation and single nucleotide variation that exist in the normal human population have made it a common practice to compare tumors to normal tissues of the same patient, in order to exclude pre-existing mutations from further analyses [39]. Therefore, the driver genomic aberrations are usually the ones that arise de novo during the early stages of tumorigenesis.

In an analogy to cancer development, driver aberrations in hPSCs should not be the ones present in the early embryo or in the fibroblast population of origin, and should be detected at relatively early culture stages, in which the genome is still stable (and thus the genetic background is still relatively quiet) (Fig. 1). Indeed, driver aberrations identified in hPSCs, such as trisomy 12 or the amplification of 20q11.21, are almost always generated de novo, and can be detected as sole aberrations in some early-passage hPSC lines [20,21]. With prolonged culture propagation, these aberrant hPSC cultures tend to acquire more and more aberrations, often leading to a complex karyotype at late passages [19,21].

3.1.3. Driver aberrations are expected to accumulate rather than disappear

The genomic integrity of hPSCs is dynamic, so that every snap shot is expected to capture a few irrelevant aberrations in culture. In fact, most of the aberrations that exist in early-passage cultures are transient, and are doomed to disappear, presumably due to lack of any adaptive value [16,28]. Naturally, driver mutations are expected to accumulate in culture, and not to disappear from it (Fig. 1). Therefore, for the identification of driver aberrations one should ideally analyze hPSC cultures that have already undergone sufficient culture propagation to eliminate disadvantageous aberrations and acquire advantageous ones, but have not yet become genomically unstable.

3.2. Identification and prioritization of candidate driver genes

Once a genomic aberration is determined to be a driver aberration, the natural next goal is to uncover the driver gene(s) underlying this aberration. As the experimental validation of candidate driver genes may be time-consuming and laborious, it is important to prioritize the candidate gene list, so that efforts are first dedicated to the examination of the more likely driver genes. The considerations for this prioritization are presented in the current section. Importantly, the "driver changes" do not necessarily involve protein-coding genes, but may very well occur in "non-genes", such as micro-RNAs and/or lncRNAs; the term "gene" is thus conveniently used in this section to denote any functional DNA sequence.

3.2.1. Driver genes are expected to reside within the minimal recurrent aberration

One useful way to narrow down the list of candidate driver genes is to narrow down the size of the involved genomic aberration (Fig. 2A). Genomic aberrations may arise due to a variety of cellular defects, such as chromosome mis-segregation, aberrant repair of DNA breaks, and faulty activation of cell cycle checkpoints (reviewed in [15]). Consequently, similar aberrations — presumably driven by the same gene(s) — may considerably differ in their size and in their exact genomic location; driver genes are expected to be included in the minimal amplified region.

Demonstrating this point, gains in chromosomes 12 and 17 often involve the entire chromosome, but often encompass only one chromosome arm (12p or 17q) [19,21]. Comparison of chromosomal aberrations from multiple hPSC lines with gains in chromosome 12 further focused the aberrant genomic locus, revealing that the minimal recurrently aberrant region (or recurrent amplicon) involves the distal tip of chromosome 12 [21]. Consequently, two hallmark pluripotency genes that reside within this region, *NANOG* and *GDF3*, were suggested as candidate driver genes underlying this common aberration [21]. By studying genomic changes at a higher resolution, another study could further narrow down this aberration, identifying as the smallest common duplicated region a genomic locus that encompasses only two genes, *NANOGP1* and *SLC2A3* [29]. This intriguing finding could suggest

Fig. 2. Guidelines for the identification and prioritization of driver genes. (A) Driver genes are expected to reside within the minimal recurrent aberration. For example, *BIRC5* (*Survivin*) resides within a recurrently amplified region in the long arm of chromosome 17. (B) Inter-species comparisons can pinpoint candidate driver genes. Ortholog genes that reside within a conserved aberration make good candidates. For example, *Birc5* resides within a recurrently amplified region in mouse chromosome 11, which is syntenic to the aberrant region in human chromosome 17. (C) Driver genes are expected to be differential, so that genes that reside within an amplified region should be significantly over-expressed across aberrant cell lines. For example, *BIRC5* is over-expressed both in human and in mouse aberrant cells. Other genes in this region are presented for reference. (D) Driver genes are expected to have functional roles in relevant cellular processes, often involved in tumorigenesis as well. Genes known to increase self-renewal, to confer resistance to apoptosis or to increase replication and/or proliferation make good candidates. *BIRC5* is a known anti-apoptotic gene. (E) Once candidate driver genes are revealed, genetic manipulation experiments are required to validate their role in the aberration.

a potential unknown role to the pseudo-gene *NANOGP1*; however, as *NANOG* itself resides just outside this region, and is included in many of the aberrations, it may also be that an upstream *NANOG* control element is present in the duplicated region [29].

Similarly, trisomy 20 is sometimes detected in hPSC cultures, but the local amplification of the 20q11.21 region is much more prevalent [20]. Implementing the same reduction strategy, a minimal amplicon of 0.55 Mb was identified, encompassing only three genes that are expressed in hPSCs: *HM13*, *ID1* and *BCL2L1* [20,31]. This short list could then be subjected to experimental validation, revealing the anti-apoptotic *BCL-XL* variant of *BCL2L1* as a true driver gene [31,32].

3.2.2. Inter-species comparisons can pinpoint candidate driver genes

An interesting approach to uncover candidate driver genes is the comparison of genomic aberrations between hPSCs of various species (Fig. 2B). The gain of chromosome 17 or of its long arm 17q is very common in hPSC cultures [21,23]. However, applying the "minimal recurrent amplicon" approach was insufficient to uncover driver genes, as this region (17q25) encompasses multiple genes [19,21]. A recent study reported that the most common chromosomal aberration in mouse PSCs is the gain of chromosome 11, or of its distal tip 11qE2. Interestingly, the minimal mouse recurrently aberrant region 11qE2 is completely syntenic to the minimal human recurrently aberrant region 17q25 [26]. A comparison of orthologous genes yielded a list of 7 such genes that reside within the minimal aberrant region and are significantly over-expressed in both species [26]. These results reiterated the potential importance of the anti-apoptotic gene *BIRC5* (also known as *Survivin*) as a candidate driver gene in chromosome 17 aberrations [19,26]. Moreover, this comparison also uncovered novel candidate genes, which had not been previously connected to trisomy 17 [26]. A similar comparison of CNAs between mouse and human PSCs, not reported to date, may similarly yield novel candidate genes, and is thus warranted.

It is important to note, however, that genomic aberrations are often not evolutionarily-conserved [26]. Some driver genes may therefore be species-specific, as a result of their unique chromosomal context, or due to the exact culture conditions and developmental stage that characterize each species' hPSCs. Interestingly, the chromosomal aberrations that arise in mouse epiblast-like pluripotent cells (EpiSCs) are not syntenic to the ones observed in hPSCs, despite the similar developmental stages of these cell types [26]. It remains to be seen whether naïve hPSCs would acquire chromosomal aberrations syntenic to the ones prevalent in mouse PSCs.

3.2.3. Driver genes are expected to be differentially expressed

A third guideline for driver gene identification and prioritization is that these genes should actually be differentially expressed in the aberrant hPSCs, compared to their diploid counterparts (Fig. 2C). Careful analyses of aneuploidies in cancer and in hPSCs showed that only a minority of the genes within an aberrant region are differentially expressed in a consistent, significant manner [21,40,41]. Several explanations may be provided for this observation: the dependence of some genes on transcription factors encoded from other chromosomes (that is, the existence of other limiting factors); the "overload" of the transcription machinery due to the imbalanced genomic content; or the existence of a yet-unidentified cellular compensation mechanism that keeps the expression of certain genes within limited boundaries.

In any case, for a driver gene to execute its function, we expect it to be actually over- or under-expressed (depending on the type of aberration, of course) in the aberrant cell lines. For example, the expression level of *NANOG*, but not of *NANOGP1*, was found to be elevated in hPSCs with trisomy 12, possibly hinting towards the former as the driver gene of this aberration [21]. Applying the "differential expression" criterion to the syntenic region identified between human chromosome 17 and mouse chromosome 11 enabled to narrow down the list of candidate genes, further highlighting the potential involvement of *BIRC5* in this recurrent aberration.

3.2.4. Driver genes are expected to be functionally important for relevant cellular processes

Complementing the abovementioned approaches, an essential requirement from driver genes is that they would actually be able to "drive" the occurrence of the aberration at hand. In other words, candidate genes should also be considered according to the likeliness that they confer a selection advantage to cells that differentially express them. A key question would therefore be: what are the potential mechanisms to confer a selection advantage to hPSCs? Again, a comparison to the mechanisms involved in cancer transformation could come in handy (Fig. 2D). The fact that pluripotency genes are often involved in cancer [7], and that key cancer genes are involved in pluripotency induction and maintenance [4], lends support to such an approach. Moreover, the findings that two of the most recurrent aberrations in hPSCs, trisomy 12 and the gain of 20q11.21, increase the tumorigenicity of hPSCs and lead to profound cancer-like changes in their molecular and cellular phenotypes [25,42–44] make an appealing argument for the involvement of cancer genes in culture adaptation. Indeed, all of the candidate driver genes described hereinafter (*NANOG*, *BIRC5*, *BCL2L1*, *AR* and *ICT1*) have been implicated in some kinds of cancer [45–49].

One mechanism to confer a selection advantage to hPSCs involves the perturbation of the delicate balance between self-renewal and differentiation [50]. Pluripotency factors can shift this balance towards self-renewal [51], are often over-expressed in cancer [7], and their over-expression may render pluripotent cells less susceptible to differentiation [52]. The over-expression of *NANOG*, a hallmark gene of the core pluripotency network, in hPSCs with trisomy 12 [21] may thus divert this balance towards self-renewal and consequently jeopardize differentiation.

A second mechanism to confer a selection advantage is the inhibition of apoptosis. hPSCs are highly sensitive to apoptosis, which is readily induced upon genotoxic and other insults [53,54]. Therefore, any inhibition of the apoptosis process is expected to confer a significant growth advantage to these cells, and would thus be selected for. Indeed, the anti-apoptotic gene *BIRC5*, suggested as a candidate gene for the recurrent gains in chromosome 17, was shown to be essential for hPSC viability and for their ability to form tumors [55,56]. Recently, the anti-apoptotic variant of *BCL2L1*, called *BCL-XL*, was validated as a true driver gene in the recurrent CNA of 20q11.21 [31,32].

A third mechanism to confer a selection advantage is an increased proliferation rate. Recurrent aberrations in hPSCs may increase their proliferation, by increasing their replication or by inducing changes in their cell cycle profile [25,44]. Naturally, differential expression of genes that affects proliferation is expected to be of high adaptive value, and cells that proliferate faster will be able to outcompete their slow-replicating counterparts in the culture. Genes involved in cell cycle progression, such as the androgen receptor (AR) on chromosome X, have thus been suggested as candidate driver genes [19].

Finally, novel candidate driver genes may be suggested based on their involvement in cancer, even if their exact mechanism of action is yet unknown. Immature colon carcinoma transcript 1 (*ICT1*) is essential for cell viability of some cancer cell lines [49], is highly expressed in undifferentiated hPSCs and in immature colon carcinoma cells [26,57], and may be regulated by pluripotency factors [26]. It resides within the minimal amplicon of human chromosome 17 and mouse chromosome 11, and is upregulated in the aberrant cells of both species [26]. It therefore makes a strong candidate gene that may underlie this common aberration (potentially, together with *BIRC5*).

3.3. Experimental validation of candidate aberrations and genes

3.3.1. Validating candidate driver aberrations

It is sometimes thought that the fact that an aberration has undergone positive selection in culture is sufficient for it to be considered a true driver aberration. Indeed, the high prevalence of some aberrations in clonal hPSC cultures strongly suggests that these aberrations introduce a selection advantage. However, it is advisable not to pre-assume

what needs to be proven. Some aberrations may depend on previous ones (i.e., arise only on the background of existing aberrations), making them secondary only despite their prevalence [26]. Moreover, as the resolution of detection is always limited to the applied methodology, existent driver mutations may exist below the detection threshold (for example, a point mutation may facilitate a chromosomal aberration). Lastly, non-genetic changes that mediate specific aberrations may accumulate during culture adaptation, leading to the fixation of neutral aberrations.

How, then, can the driving role of a recurrent aberration be demonstrated? One possibility is to directly examine suspected genes within this recurrent aberration, inferring the driver nature of an aberration by revealing a driver gene within it [31]. Another possibility is to derive and study hPSCs with congenital aberrations; if cell lines that harbor a congenital aberration identical to that arising from culture adaptation, also exhibit the same advantageous cellular phenotype, this would be a strong indication for the driver role of this aberration [25]. Finally, introduction of an entire genomic region that is recurrently amplified in hPSCs, using plasmids or BACs, may facilitate the study of that recurrent aberration, enabling the informative comparison of isogenic same-passage clones.

3.3.2. Validating candidate driver genes

Up until recently, several limitations impeded the validation of driver genes underlying recurrent genomic aberrations in hPSCs. The lack of confirmed "hotspots" (or minimal amplicons, as discussed above), the difficulty to prioritize candidates for experimental validation, and the lack of reported distinguished phenotypes in aberrant cells, made it very challenging to pinpoint and confirm the driver genes. Of note, although the demonstration of a correlation between differential expression of specific genes to the gradual takeover of an aberration in a mosaic culture was an important step forward [21], it should not be considered a validation, as it merely reveals a correlative, rather than a causative, link.

Two recent papers have successfully overcome these obstacles and experimentally validated for the first time a driver gene in a recurrent aberration. Starting from a minimal shortlist of only 3 or 4 genes that reside within 20q11.21, a minimal genomic region often amplified in hPSCs and in human embryonal carcinoma cell lines, these studies focused on the anti-apoptotic gene *BCL2L1* [31,32]. The anti-apoptotic protein variant of this gene, *BCL-XL*, was significantly over-expressed in the aberrant hPSCs, conferring a resistance to apoptosis that resulted in an increased growth rate of the cells. With this characterized phenotype at hand, these studies could demonstrate that an over-expression of this gene in WT hPSCs provides them with growth characteristics similar to those of the aberrant cells, whereas its knockdown in aberrant cells suppresses this growth advantage [31,32].

While over-expression and knockdown experiments are surely useful (Fig. 2E), one potential caveat still exists. Genomic aberrations often involve a single extra copy of a genomic region, and rarely involve more than 3 or 4 extra copies [21,31]. Over-expression and knockdown experiments, on the other hand, may yield an order-of-magnitude-higher change in protein expression. Such a significant over-expression of an anti-apoptotic protein could lead to a considerable reduction in apoptosis and a strong selection advantage regardless of whether this gene is the real driver of the examined aberration. It is therefore important to make sure that the applied genetic manipulations result in protein levels that are comparable to those of the WT and aberrant cells. This could be done by fine-tuning the protein levels induced by RNAi [31], by inserting a gene under its endogenous promoter, or by targeting one allele of a gene using the rapidly-developing genome editing techniques (e.g., CRISP-Cas9) [58].

4. Concluding remarks

In a classic paper published in 1943, Luria and Delbrück formulated a theoretical framework to analyze the appearance of adaptive mutations in bacterial populations [59]. By experimentally studying the distribution and dynamics of bacterial populations exposed to viruses, they were able to reach the conclusion that resistant bacteria arise by mutations that occur independently of the action of the virus [59]. This landmark work has greatly influenced the field of genetics, as it demonstrated that the Darwinian forces of random mutation and natural selection come into play in (bacterial) cells cultured in vitro. A few decades later, following the derivation of human cell lines [60], it soon became evident that this is a general principle: cellular heterogeneity and cell selection result in adaptive mutations in practically all populations of culture-propagated cells.

Recent years have seen a plethora of descriptive studies that characterized in details the genomic aberrations in hPSCs. These studies have greatly contributed to an increasing understanding of the SNAs, CNAs and chromosomal aberrations that characterize hPSCs, as well as their potential cellular origins. However, to gain a valuable understanding of the underlying mechanisms, it will be necessary to understand the cellular ramifications of recurrent aberrations, as well as the molecular mechanisms that facilitate their formation. Highly pertinent to that issue is the separation of driver aberrations from irrelevant passenger aberrations, and the identification of driver genes whose differential expression explains the strong selection advantage of recurrent aberrations.

In this review, I have described the genes currently known to be involved in common hPSC genomic aberrations of all kinds, outlined guidelines for the identification and prioritization of candidate driver aberrations and genes, and discussed potential ways to validate these candidates experimentally. A common theme emerging from this discussion is the utility of harnessing terminologies and methodologies of cancer research to the study of genomic integrity in hPSCs. In the future, lessons from cancer research may become even more relevant for the development of new in-silico, in-vitro and in-vivo techniques to identify, prioritize and experimentally validate driver aberrations and driver genes. Novel findings on genes that underlie genomic aberrations in hPSCs may in turn yield insights of equal importance for understanding genomic instability in cancer, thereby closing a fruitful scientific circle.

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