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News & Views

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Too much to differentiate: aneuploidy promotes proliferation and teratoma formation in embryonic stem cells

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Aneuploidy, or an uneven number of chromosomes, has mostly detrimental consequences in eukaryotic cells, which include impaired proliferation as well as compromised DNA replication and protein folding. Unexpectedly, a new study published in this issue of *The EMBO Journal* shows that in murine embryonic stem cells aneuploidy does not interfere with proliferation, but rather hinders their differentiation capacity, thus propelling the formation of poorly differentiated teratomas.

See also: **M Zhang *et al*** (November 2016)

Maintaining genome integrity is an essential task for every cell and there are many ways in which things can go wrong. One of the most frequent errors affecting genome integrity is chromosome missegregation, which occurs at an estimated frequency of 1:1,000 in mitosis and even more often during meiosis. In the immediate response to chromosome segregation errors, most cells will irreversibly arrest, but surviving cells can give rise to an aneuploid population. Aneuploidy, a cellular state characterized by an unbalanced number of chromosomes, is the leading cause of spontaneous miscarriages and a hallmark of cancer. Yet, what makes aneuploidy so detrimental to cellular physiology has been difficult to tackle.

Recent years have witnessed an increased interest in aneuploidy fueled to a great degree by the establishment of novel cell models with simple defined aneuploidies. Over the past years, model systems from yeast, *Drosophila, Arabidopsis* as well as murine and human somatic cells have been established that gained a part or a whole chromosome and their phenotypes and physiology have been analyzed in comparison with their isogenic euploid counterparts. Most studies using these model systems revealed that a gain of even a single chromosome impairs proliferation and leads to globally altered gene expression, proteotoxic stress, elevated genomic instability, and differential drug sensitivity (for a recent review, see Santaguida and Amon, 2015). While the underlying molecular mechanisms of these complex changes remain poorly understood, two striking features became apparent: i) there is a marked similarity in the cellular response to aneuploidy across species regardless of the identity of the extra chromosome, and ii) the findings fail to provide a plausible explanation for whether and how exactly aneuploidy *per se* promotes cancer.

All the above-mentioned results were obtained with somatic aneuploid cells. The consequences of aneuploidy in stem cells, which differ from somatic cells in many aspects, have not been systematically investigated. In a study published in this issue of *The EMBO Journal*, Meili Zhang and coworkers characterized multiple aneuploid cell lines derived from murine embryonic stem cells. Using PiggyBac-based DNA vectors that are both positively and negatively selectable based on their copy numbers, the authors were able to create a series of murine embryonic stems cells trisomic for chromosome 6, 8, 11, 12, or 15. Thanks to the used selection cassette, these cells can be easily corrected to diploidy, which provides closely matched control cell lines. Remarkably, four out of five trisomic ES cell lines showed normal or higher proliferation rates and formed more colonies then diploid controls. These colonies expressed alkaline phosphatase and pluripotency markers, which demonstrates that they have maintained their stemness. Moreover, spontaneous differentiation was delayed and inefficient in trisomic cells and their lineage commitment was impaired. Upon subcutaneous injection into immunodeficient SCID mouse, trisomic cells created poorly differentiated teratomas and teratocarcinomas faster than diploids and displayed increased tumor weight.

To determine the reasons for the differentiation defects, the authors analyzed gene expression and identified uniform global transcriptional changes independent of the type of the extra chromosome. The pattern of gene expression changes was different to what has previously been observed in somatic aneuploids (e.g., Sheltzer, 2013; Dürrbaum *et al*, 2014). The only pathways that seem to be deregulated similarly in both somatic and stem cells were related to extracellular region and matrix, although different genes were affected in the different cell types. In striking contrast to the aneuploid somatic cells, no downregulation of replication- and cell cycle-related pathways was observed. This observation is in

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agreement with the absence of a proliferation defect in aneuploid ES cells in the current study. Instead, several pathways related to development were significantly downregulated, among them neural plate development, BMP signaling, Fgf receptor signaling, and others linked in particular to primed pluripotency and differentiation-associated processes.

The authors hypothesized that the increased tumorigenic potential of the trisomic cells might be due to both the increased proliferative potential combined with reduced differentiation capacity. Interestingly, the lack of differentiation in aneuploid cells was not due to changes in DNA and histone methylation status of aneuploid cells. Instead, the impaired differentiation of trisomic cells could be rescued by culturing in a mixture with diploid ES cells or by the addition of conditioned media. Consistently, addition of extracellular differentiation factor BMP4 was sufficient to partially complement the differentiation defects of aneuploidy ES cells.

These findings support previous observations obtained with ES cell lines with trisomy 21 that revealed in various settings that chromosome gain interferes with differentiation (e.g., Roy *et al*, 2012). Similarly, spontaneously arising human stem cells with trisomy of chromosome 12 (T12-hPSC) grew faster in culture, showed increased replication and gave rise to more aggressive teratomas and teratocarcinomas from which pluripotent cells could be recovered, suggesting that the cells remained largely undifferentiated (Ben-David *et al*, 2014). In contrast, proliferation capacity was strongly impaired in aneuploid murine hematopoietic stem cells that were either trisomic for a specific chromosome or chromosomally unstable due to increased chromosome missegregation rates caused by mutation in spindle assembly checkpoint gene Bub1 (Pfau *et al*, 2016). Similar results were obtained in *Drosophila* cells where aneuploidy was induced by interference with centrosome duplication and spindle assembly checkpoint by overexpressing Sak (the Plk4 ortholog) in combination with a deletion of the spindle assembly checkpoint protein Mad2 (Gogendeau *et al*, 2015). Here, aneuploidy in neural stem cells and adult intestinal stem cells reduces the number of proliferative cells mainly due to an extended G1 phase, which leads to cell cycle exit, premature differentiation, and decreased tumorigenic potential.

The results obtained *in vitro* using various cell types and species demonstrate convincingly that the cellular response to aneuploidy is largely independent of the type of aneuploidy and the identity of the altered chromosomes. Instead, the response is highly cell type specific and surprisingly complex. While aneuploid somatic cells and somatic stem cells experience a series of detrimental consequences that impede their proliferation and slow down tumor formation (Gogendeau *et al*, 2015; Sheltzer *et al*, 2016), embryonic stem cells appear to proliferate well and promote tumor formation even with an extra chromosome (Fig 1). These observations are reminiscent of those obtained with *in vivo* mouse models developed to study the role of chromosomal instability and aneuploidy in tumorigenesis that revealed that aneuploidy may act both as an oncogene and a tumor suppressor (e.g., Weaver *et al*, 2007). The presented work might provide new insight into the conundrum of how aneuploidy can promote carcinogenesis despite having anti-proliferative effects in somatic cells. In the future, it will be essential to understand why the ES cells respond to aneuploidy so differently.

Stem cells hold huge promise for regenerative medicine and serve as a model system for studying diseases and developing new drugs. While aneuploid cells seem to accumulate in various somatic cell types in the aging mouse, aneuploidy in stem cell lineages in the same mice remains rare, indicating that mechanisms protecting against numerical chromosome abnormalities are efficient in stem cells. Yet, due to so far unknown reasons trisomies often arise spontaneously in *in vitro* stem cell cultures. This frequent spontaneous aneuploidy represents a substantial risk for future therapeutic applications. Additional studies and novel models of aneuploidy will therefore be essential to better understand how aneuploidy arises in *in vitro* stem cell cultures and to elucidate the consequences for the physiology and function of stem cells under different conditions.

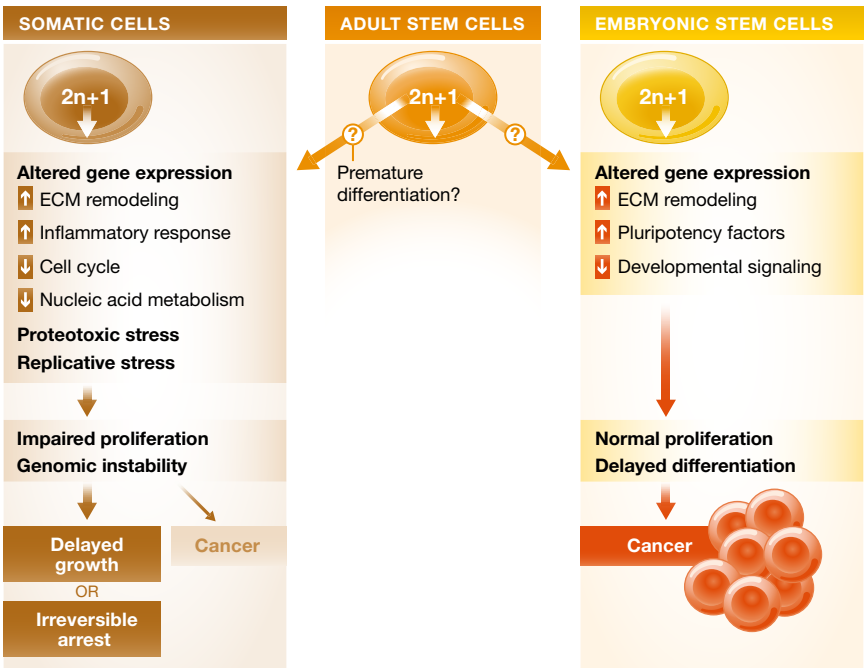

Figure 1. Aneuploidy in embryonic stem cells results in increased proliferation and impaired differentiation, a combination that promotes cancer.
As shown in the left, somatic cells display altered gene expression that negatively affects genes required for cell cycle progression as well as proteotoxic stress and impaired protein folding in response to aneuploidy. This response is conserved among species and usually leads to proliferative disadvantage and genomic instability. Embryonic stem cells exert different gene expression changes that affect extracellular matrix and developmental signaling upon gain of chromosome 6, 8, 11, 12 or 15, as shown by Zhang *et al* (2016). In contrast to somatic cells, aneuploid ES cells maintain proliferative capacity and induce tumorigenesis *in vivo*. ECM, extracellular matrix.

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