OUTLOOK

Solving the chromosome puzzle of aneuploidy in cancer

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Chromosome instability (CIN) and aneuploidy are hallmarks of cancer cells, typically associated with aggressiveness and poor outcomes. Historically, the causative link between aneuploidy and cancer has been difficult to study due to its intrinsic complexity and the poor fitness of aneuploid cells. In this issue of *Genes & Development*, two companion papers (Trakala and colleagues [pp. 1079–1092] and Shoshani and colleagues [pp. 1093–1108]) exploited sophisticated mouse models to study the progression of aneuploidy from early phases to established tumors. Both groups observed that, while in the early nontumoral cells aneuploidy is characterized by random chromosomal gains, established tumors display a stereotypic karyotype with recurrent gains of only a few chromosomes. Thus, aneuploidy in tumors is not random but shows reproducible patterns of chromosomal changes induced by mechanisms that these two studies are beginning to unveil.

Aneuploidy, the abnormality in number and structure of chromosomes in cells, is a very frequent feature of most human cancers (Taylor et al. 2018). High levels of aneuploidy are associated with several parameters of aggressiveness in cancers, including poor prognosis, metastatic spread, and resistance to therapy (Weaver and Cleveland 2007; Thompson and Compton 2008). While Theodor Boveri, with contributions from his wife Marcella O'Grady, is recognized as the first investigator to propose aneuploidy as a potential genetic cause for cancer development and laid the basis for the "chromosome theory of cancer" more than a century ago in 1914 (Hansford and Huntsman 2014), observations by David Hansemann hypothesized a connection between aneuploidy and cancer as early as the end of the 19th century (Bignold et al. 2006).

Despite this long-standing supposed connection with cancer, direct evidence that aneuploidy can cause cancer development has been difficult to obtain due to experimental complexities. Aneuploidy originates from chromosome instability (CIN) due to failure of the mitotic checkpoint that results in missegregation of chromosomes leading to gains of oncogenes or loss of tumor suppressors (Weaver and Cleveland 2007; Thompson and Compton 2008). Aneuploidy is poorly tolerated in normal cells, and cell fitness of aneuploid cells is at a disadvantage compared with euploid cells in vivo (Pfau et al. 2016). Consistently, mice with germline mutations of the proteins that control the mitotic checkpoint are typically embryonic lethal. This fitness paradox, in which aneuploidy could both drive or inhibit cancer development (Weaver and Cleveland 2007), might explain why mutations in genes involved in chromosome segregation are very rare in cancers (Duijf and Benezra 2013). In this context, it is likely that the protumorigenic or antitumorigenic effects of CIN may be related to the severity, timing, and duration —transient versus chronic—of CIN, the tissue type (Hoevenaar et al. 2020), or the tumor microenvironment, including the immune clearance of aneuploid cells (Santaguida et al. 2017). Being heterogenous in nature and involving hundreds of genes on each aberrant chromosome, the precise patterns and mechanisms of development of selective chromosomal gains or loss that link aneuploidy to cancer development have been elusive to determine. While chromosomal aberrations appear to be random in most cancers, there are cancers in which chromosomal changes show recurrent patterns of gains in selected chromosomes, such as in B lymphoblastic leukemia (Molina et al. 2021).

In this issue of *Genes & Development*, two studies provide elegant experimental evidence to help solve this puzzle by generating novel mouse models of CIN and analyzing early and late stages of aneuploidy associated with tumor development. In the work from the late Angelika Amon's laboratory, Trakala et al. (2021) induced CIN by generating a LOH (loss of heterozygosity) mouse model with a mutant *CDC20* allele ($CDC20^{AAA}$) and a conditional wild-type *CDC20* allele to be deleted in adult mice to overcome embryonic lethality. Adult cells expressing only the $CDC20^{AAA}$ protein develop CIN due to the

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inability to activate the spindle assembly checkpoint (SAC) by preventing mutant CDC20 binding and sequestration by MAD2 at the unattached kinetochores. In a companion work, Don Cleveland's group (Shoshani et al. 2021) analyzed models of transient and chronic CIN. To achieve transient CIN, Shoshani et al. (2021) developed mice with doxycycline-inducible overexpression of the Polo-like kinase 4 (Plk4) that leads to centrosome amplification. They further made mouse strains that had lost either one or both copies of p53 (PRG5 mice) and compared the type of aneuploidy with a model of chronic CIN achieved by conditional deletion of MAD2 and p53.

Quite remarkably, these mouse models showed very similar and concordant results despite their different genetic backgrounds. The induction of CIN in $CDC20^{AAA}$ mice was associated with an early phase of aneuploidy with random chromosomal gains or losses of most chromosomes, with gains being more prevalent than losses, and slight differences in chromosome changes between tissues (Trakala et al. 2021). Likewise, transient CIN in PRG5 mice was associated with random chromosome gains in thymic cells detectable for a few weeks after Plk4 induction, with return to normal karyotype within 1 mo after doxycycline withdrawal (Shoshani et al. 2021). Over time, mice developed thymic lymphoma in both models. The latency was ~9 mo in $CDC20^{AAA}$ mice, which was further reduced to 6 mo in mice with one copy loss of *p53* (Trakala et al. 2021). In PRG5 mice, thymic lymphoma development secondary to transient CIN was dependent on the loss of one or two copies of *p53* (Shoshani et al. 2021). Cancer development and aneuploidy were studied by whole-genome and single-cell sequencing and was associated with a progressive selection of a few tumor clones that not only showed a tendency for inactivation of the residual *p53* WT allele but harbored frequent and recurrent gains of selective chromosomes. Strikingly, these chromosomal changes were remarkably similar in all models. In $CDC20^{AAA}$ mice, Trakala et al. (2021) observed frequent gains of chromosomes 14 and 15 in p53 WT mice associated with additional gains of chromosomes 4 and 11 in mice with p53 loss. Likewise, Shoshani et al. (2021) found defined transcriptome and aneuploidy profiles in transient CIN models, including recurrent gains of chromosomes 4, 5, 14, and 15. Similar aneuploidy profiles were also found in a third model of chronic CIN induced by genetic inactivation of *MAD2* and *p53* that showed gains of chromosomes 4, 5, 14, 15, and 17 (Shoshani et al. 2021). Overall, both studies clearly showed an evolution of aneuploidy that appears to be largely independent of the genetic type of CIN: a transition from random chromosomal alterations, mostly gains with few losses, to early selection of aneuploid clones displaying a quite reproducible pattern of chromosomal gains limited to few selected chromosomes.

The obvious next question is: What is so special about those chromosomes that are recurrently gained? Which are the genes that actually drive clonal expansion and growth advantage in tumors? Both studies attempted to answer these difficult questions because of the high number of potentially causative genes. By expression profile analysis, both groups identified MYC, and genes under the MYC transcriptional control, as being recurrently up-regulated, consistent with copy number gains of *MYC* on chromosome 15. Very elegantly, Trakala et al. (2021) demonstrated that *MYC* is likely one of the key genes in this process because they also observed frequent gains of chromosome 6 when an extra copy of *MYC* was introduced on this chromosome, indicating that the position of the *MYC* gene in a chromosome is sufficient to determine the stereotypical tumor karyotype. Still, the puzzle is likely more complex, as additional genes could drive the selective gains of chromosome 15, such as the *Rad21* gene studied by Trakala et al. (2021), while several other genes could likely be responsible for the recurrent gains of the other chromosomes: 4, 5, and 14. Thus, we are just at the beginning of the process of solving an intricate puzzle that connects chromosomal changes, aneuploidy, and tumor development. In this complicated path, the studies by Trakala et al. (2021) and Shoshani et al. (2021) provide important tools and highlight reproducible patterns that shed a first initial light to guide future discoveries.

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