CellPress

Spotlight

The KaryoCreate technology generates specific aneuploid karyotypes on demand

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https://doi.org/10.1016/j.crmeth.2023.100514

In a recent issue of *Cell*, Bosco et al. present an innovative methodology named KaryoCreate that allows the generation of chromosome-specific aneuploidy in human cells in order to investigate the ontogenesis and the multifaceted aspects of aneuploidy in physio-pathological contexts.

Aneuploidy is a pathological condition that originates from errors during cell division when the resulting daughter cells inherit an unbalanced karyotype.[1] Historically, the term referred to the presence of supernumerary or inferior copies of whole chromosomes, but with the advent of high-resolution molecular tools to study the genome, a variety of chromosome rearrangements have been observed, resulting in structural aneuploidy. These rearrangements are either balanced, if the complete chromosome set is still present (i.e., inverted or translocated chromosomal regions), or unbalanced, if it has additional or missing information (i.e., deletions, duplications, insertions, or isochromosomes).

Accurate segregation of chromosomes relies on the proper temporal and spatial attachment of the spindle apparatus to the chromosomes via a protein complex named kinetochore in which the centromere plays a central role by serving as docking site for its assembly.[2] Proper spindle–kinetochore attachment and chromosome movements are then controlled by the spindle assembly checkpoint (SAC) and the chromosomal passenger complex (CPC). Chromosome mis-segregation can arise at multiple levels such as from improper kinetochore formation or spindle microtubules attachment to the kinetochores leading to numerical and structural aneuploidy. In this context, altered expression or mutation of mitotic checkpoint components are found in a subset of aneuploid human cancers.[2]

Aneuploidy is mostly incompatible with life and is a hallmark of numerous pathological conditions in humans. It is recognized as the most common genetic abnormality resulting in embryonic demise, pregnancy loss, and congenital birth defects,[3] and it is prevalent in cancers, where 80% of solid tumors and 60% of hematological neoplasms are aneuploid. Aneuploidy is believed to drive cell transformation by fueling chromosomal instability in order to confer evolutionary growth advantage and cellular adaptation.[1] Although aneuploidy is a hallmark of cancer and appears to be instrumental to shape the complexity of cancer genomes, the mechanistic onset and evolution of chromosomal changes in tumorigenesis is still poorly understood. Over the years, the idea that aneuploidy plays a causal role in the origin of cancer has been primarily supported by the discovery that certain tumor types present a characteristic aneuploidy pattern. For example, trisomy 12 is the most common cytogenetic abnormality in chronic lymphocytic leukemia, and chromosome 18q is lost in about 62% of colorectal cancer patients, with the gain of chromosome 13 occurring almost exclusively in this tumor type. Certain cancers have conserved characteristics of structural aneuploidy as in the case of chronic myelogenous leukemia, in which a chimeric gene is observed as a result of a translocation between chromosomes 9 and 22.[4] Thus, aneuploidy and cancer are two closely related phenomena, and several theories have been put forward to explain the etiology of this relationship. For example, whole-chromosome aneuploidy may provide cancer cells with a mechanism that causes loss of tumor suppressor genes or amplifications of oncogenic loci or with a tolerance pathway able to compensate the loss or gain of genetic material.[5]

The rates of human chromosome mis-segregation vary among chromosomes with certain chromosomes that are more prone to mis-segregate than others, as observed in conditions that perturb the genome stability. At the molecular level, chromosome-specific aneuploidy can be influenced by an intrinsic heterogeneity of centromeric DNA and its binding components[6] and, at cellular level, by interphase chromosome position.[7] Understanding the molecular basis of chromosome-specific aneuploidy is crucial to delve into the genesis of cancer genome instability to design efficient therapies. However, to date, this knowledge gap is associated with the lack of appropriate mammalian models to generate specific aneuploidies.

While random aneuploidy can be easily obtained in cell models by chemically or genetically interfering with microtubule dynamics, mitotic checkpoint (SAC inhibitors), or centrosome regulation, the achievement of chromosome-specific aneuploidy is more challenging. Introducing or eliminating specific chromosomes is technically challenging due to the lack of experimental tools capable of directly perturbing segregation of a single chromosome in living cells with high efficiency. In the last decade, chromosome-specific gains and losses were achieved in mouse and human models using micro-cell-mediated chromosome transfer from a donor cell to a recipient cell,[8] Cre-Lox recombination,[9] and XIST-mediated transcription chromosome silencing.[10] These methodologies require laborious protocols and lack high efficiency, despite being currently improving to some extent. With the development of the CRISPR-Cas9
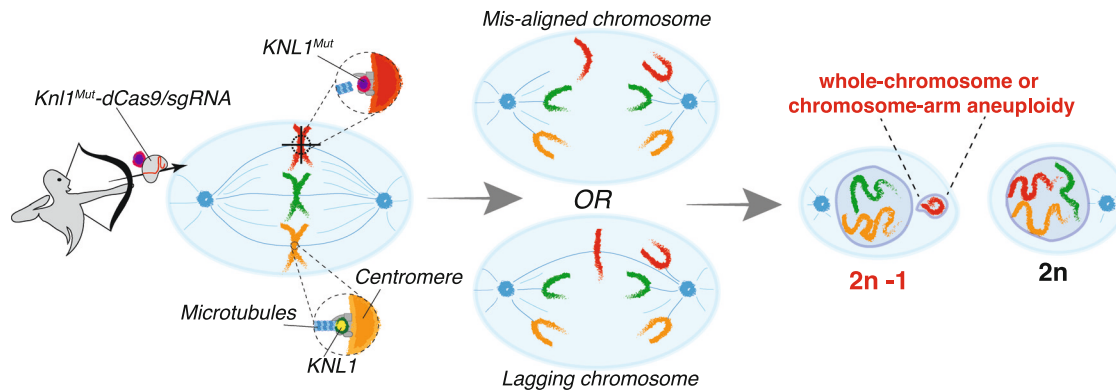
Figure 1. Schematic of the KaryoCreate to induce chromosome-specific mis-segregation in human cells
Tethering of a mutated truncated version of the kinetochore protein KNL1 (KNL1^{Mut}) to centromere-specific sequences via dCas9/gRNA interaction induces chromosome mis-segregation of the targeted chromosome leading to whole-chromosome or chromosome-arm aneuploidy.

gene-editing technology, multiple chromosome-specific double-strand breaks can be obtained by single or multiple sgRNAs targeting unique chromosome-specific sequences, therefore inducing structural aneuploidy. While this approach is more versatile, the unwanted side effect induced by Cas9 cutting (including unscheduled chromosome rearrangements) and a preferential loss of certain chromosomes over others limit the use of the technology. In this respect, the use of the nuclease-dead Cas9 (dCas9) to tether a specific protein capable of interfering with chromosome segregation held the promise to bypass these issues. This strategy was recently used to either generate an ectopic kinetochore by targeting the centromere protein CENP-T[11] or by counteracting chromosome congression via a Kinesin14VIb-mediated pulling force to chromosome-specific repetitive sequences.[12] Both these approaches perturb chromosome segregation fidelity of the target chromosomes but, due to the type of mis-segregation, result mostly exclusively in segmental chromosomal arm aneuploidies.

A strategy named KaryoCreate designed by Davoli and colleagues, published in a recent issue of *Cell*,[13] has the potential to bypass most of the problems discussed so far. Taking advantage of the complete sequence of the human genome, the authors designed specific sgRNAs targeting 19 out of 24 centromeres, with the exception of most acrocentric chromosomes. To induce chromosome mis-segregation, they identified a mutant of the kinetochore protein

KNL1 that is capable of destabilizing the kinetochore-microtubule attachment when brought to the selected centromeres via dCas9/gRNA interaction (Figure 1). The authors propose that expression of a mutated truncated version of KNL1 (KNL1$^{1-86}$ with either RVSF/AAAA or S24A;S60A mutations) acts as a sort of dominant negative protein by generating an imbalance between AuroraB within the CPC and the phosphatase PP1, finally causing a reduction in microtubule stability. However, further studies need to be done to elucidate the exact molecular mechanism of how this mutated KNL1 interferes with endogenous KNL1.

The tethering of KNL1^{Mut}-dCas9 with centromere-specific guides induces minor mitotic delay accompanied by misalignment and lagging chromosomes that end up in micronuclei formation containing the targeted chromosome, with an aneuploidy frequency around 15% (Figure 1). The success of the technology in generating gains and losses of specific human chromosomes is largely dependent on the expression levels of the KNL1Mut-dCas9 construct, the ability of the aneuploid clones to double and propagate for some generations, and the selective pressure that leads to the prevalence of a certain loss or gain of a specific chromosome.

Surprisingly, along with chromosome gain and loss, 55% of the aneuploidy events generated by KaryoCreate are whole-arm aneuploidy (60% losses and the rest are gains). How, when, and why this occurs requires deeper investigation.

It is likely that the presence of dCas9 acts as a replication blockage, causing DNA damage accumulation and a series of events leading to (peri-)centromeric breakage, with particular centromeric features that make some of them more prone to ruptures than others.[6] This might explain why the tethering of the dCas9 per se is sufficient to induce a certain level of chromosome mis-segregation (about 1/3 compared to KNL1^{Mut}-dCas9), a general issue of the dCas9-based approaches.[12] Alternatively, it is possible that KNL1Mut-dCas9 increases the chances of incorrect attachment (e.g., merotelic), as observed by the presence of lagging chromosomes, that consequently could promote rupture during chromosome pulling.

The study of specific aneuploidy in non-mutagenic contexts is the most important step to understand the causes/effects leading to a certain aneuploidy pattern, the cellular signaling pathways that are perturbed by the unbalanced karyotype, and the tumor-specific response to the chromosome-specific aneuploidy in relationship to the other genetic/epigenetic aberrations characteristic of a certain tumor. In this regard, the authors show the role of chromosome 18 loss in promoting colorectal cancer and resistance to TGFβ signaling, as proof of principle of their technology.

KaryoCreate still carries a few limitations: it induces more losses than gains and more events at arm level than whole chromosomal events, and it requires several days (at least 4 to obtain an aneuploidy frequency of 10%) to induce the

desired karyotype, therefore missing the initial events of how individual cells respond to chromosome-specific aneuploidy. It will be useful for the community to overcome these problems and to generate gRNAs able to also target the centromeric sequences of acrocentric chromosomes in order to have a broader and clearer understanding of the whole pathological context characterized by specific monosomies, trisomies, and chromosomal rearrangements. The generation of a repertoire of several cell clones characterized by a specific aneuploidy pattern can then be added to pre-existing aneuploidy cell models to enable performing large-scale chemical screens and gene expression experiments to evaluate the direct consequences of aneuploid status in therapy response.

In summary, KaryoCreate stands as tool of choice to fulfill the fundamental need to understand the causes and consequences of chromosome-specific aneuploidy, with broad applicability from *in vitro* fertilization studies to chromosomal disorders and cancer.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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