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Visualizing chromosome segregation in live cells

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Errors in chromosome segregation result in chromosome instability (CIN), which is one of the hallmarks of cancer, but paradoxically can also reduce the ability of cancer cells to proliferate. Studying this complex relationship between CIN and cancer progression has been challenging due to a lack of methods to easily monitor the segregation of individual chromosomes in real time.

Early methods to quantify chromosome missegregation included using colony sectoring assays in budding yeast,[1] eye color mosaicism in flies[2] and measurement of loss of a non-essential chicken chromosome bearing a hypoxanthine phosphoribosyltransferase (HPRT) gene in a Chinese hamster hybridoma cell line.[3] Fluorescence In-Situ Hybridization (FISH) and Spectral Karyotyping (SKY) have since allowed to look at numerical and structural alterations of chromosomes,[4] but these assays are cumbersome. More recently, advances in DNA sequencing technology have allowed the determination of chromosome ploidy in individual cells using single cell sequencing.[5] Although these approaches have produced very important information regarding the frequency of aneuploidy in different cell types and tissues, these methods are time consuming, costly and constitute an end point assay, since the cells are fixed or destroyed as part of the assays.

New approaches that use fluorescent proteins as a readout to quantify chromosome missegregation in live cells have been developed.[6,7] These approaches allow efficient quantification of chromosome missegregation in single cells using live cell microscopy and/or flow cytometry, and can be easily scalable to allow for high throughput identification of genes or drugs that affect chromosome segregation. However, an important caveat of these approaches is that the gain or loss of fluorescence that signals chromosome missegregation depends on the turnover of the fluorescent protein or shRNA employed, which in the case of the loss-of-fluorescence assay using eGFP is 14 d.[6] This slow turnover of GFP constitutes a significant limitation that precludes the analysis of chromosome missegregation in real time.

This limitation is overcome in a new assay developed by Markossian and co-workers that substitutes the stable eGFP for a short lived eGFP reporter engineered by fusing eGFP to the destruction box (DB) region of securin.[8] This region is responsible for targeting securin for ubiquitination-mediated degradation by the proteasome. Addition of the securin DB targets DB-eGFP for degradation at the metaphase to anaphase transition, ensuring that the fluorescence is reset in every cell cycle and thus allowing the evaluation of chromosome segregation after every mitotic division. In addition, the authors have combined this reporter assay with the ability to directly visualize an artificial chromosome using a Human Artificial Chromosome (HAC) containing tetO sequences that can be visualized through the binding of an mCherry-TetR protein. This dual reporter system combines the easy detection of whole cell fluorescence via the DB-eGFP with the ability to follow the segregation of an individual artificial chromosome in live cells by microscopy. This combination results in a robust system suitable for both high throughput applications as well as detailed analysis of the dynamics of chromosome segregation. This versatility will open new avenues to answering fundamental questions about chromosome segregation at multiple levels, from understanding the mechanisms involved in chromosome segregation at the molecular level, to the systematic search for drugs targeting this process.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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