A way to stabilize haploidy in animal cells

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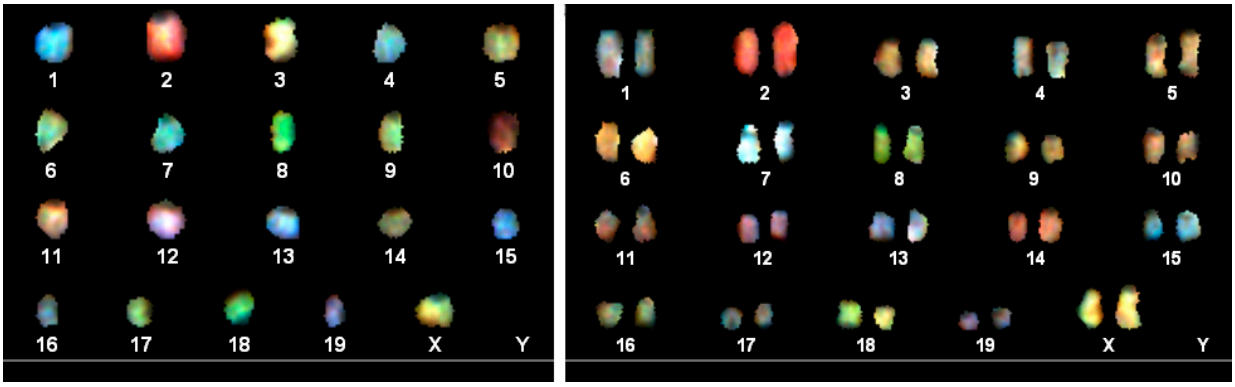

SKY analysis of haploid and diploid cells. Credit: CNIO

The emergence in recent years of the first mammalian haploid cell lines has raised great expectations in the scientific community. Despite their potential, these cultures present some issues that complicate their use because haploidy is unstable and can be lost quickly. The Genomic Instability Group at the Spanish National Cancer Research Centre (CNIO) has offered an explanation of this phenomenon and proposes a way to overcome it. This work has been published in the journal *Proceedings of the National Academy of Sciences* (*PNAS*).

With the exception of the sperm or ovules, cells contain two sets of chromosomes, one from each parent. However, organisms with a single set of chromosomes (haploids), such as yeast, are extremely useful for genetic studies and are crucial in identifying key genes and pathways.

Laboratory yeasts enabled studies on autophagy by Yoshinori Ohsumi, which earned him the Nobel Prize in Medicine in 2016, and the Nobel-winning discovery of the cell cycle regulatory genes.

"As [yeast] has only one set of chromosomes, it is very easy to find interesting mutants, as all you have to do is to alter a single allele to produce a phenotype," says Oscar Fernández-Capetillo, head of the Genomic Instability Group and the leader of the research project. "In mammals, in the absence of haploid cells, other approaches have been used to identify key genes, such as interfering RNA, but they are sub-optimal methods. All this changed five years ago when haploid cells were discovered in a leukaemia patient (KBM7 and HAP1) and with the emergence of techniques to create mammalian haploid embryonic stem cells, developed originally by Anton Wutz," continues Fernández-Capetillo.

However, the cultures of such mammalian haploid cells become diploid within a few days. This phenomenon, which has been called "diploidization," is what Fernández-Capetillo's group has been studying. Their findings suggest that the loss of haploid cells is due to their limited viability, and therefore, they are replaced by existing diploid cells in the cultures.

"When you try to isolate haploid cells, it is very difficult to take only one; you usually separate several so you always drag along a diploid. When you culture them, you invariably observe that the haploid cells die and the diploid cells become the majority," he says. "We now know that this happens because the haploid cells activate death mechanisms via p53."

Their studies show that the problem arises when the haploid cells try to separate their chromosomes during mitosis. The machinery involved in cell division has been designed to handle a fixed amount of DNA (46

chromosomes). When there is more (polyploidy) or less (haploidy), mitosis is more prone to errors during the segregation of the chromosomes, and this activates p53. This is the reason why haploid cell cultures do not thrive. By eliminating p53, as this study demonstrates, haploid cells are able to survive.

"Our findings should facilitate the use of animal haploid cells, making them accessible to a broader range of laboratories and technologies," the authors conclude. Currently, the group is trying to discover chemical forms of stabilizing haploidy in animal cells and is exploring strategies that would allow the creation of organs or even animals that only have a maternal set of chromosomes.

More information: A p53-dependent response limits the viability of mammalian haploid cells *Proceedings of the National Academy of Sciences* (2017). www.pnas.org/cgi/doi/10.1073/pnas.1705133114

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