Malignant cells adopt a different pathway for genome duplication

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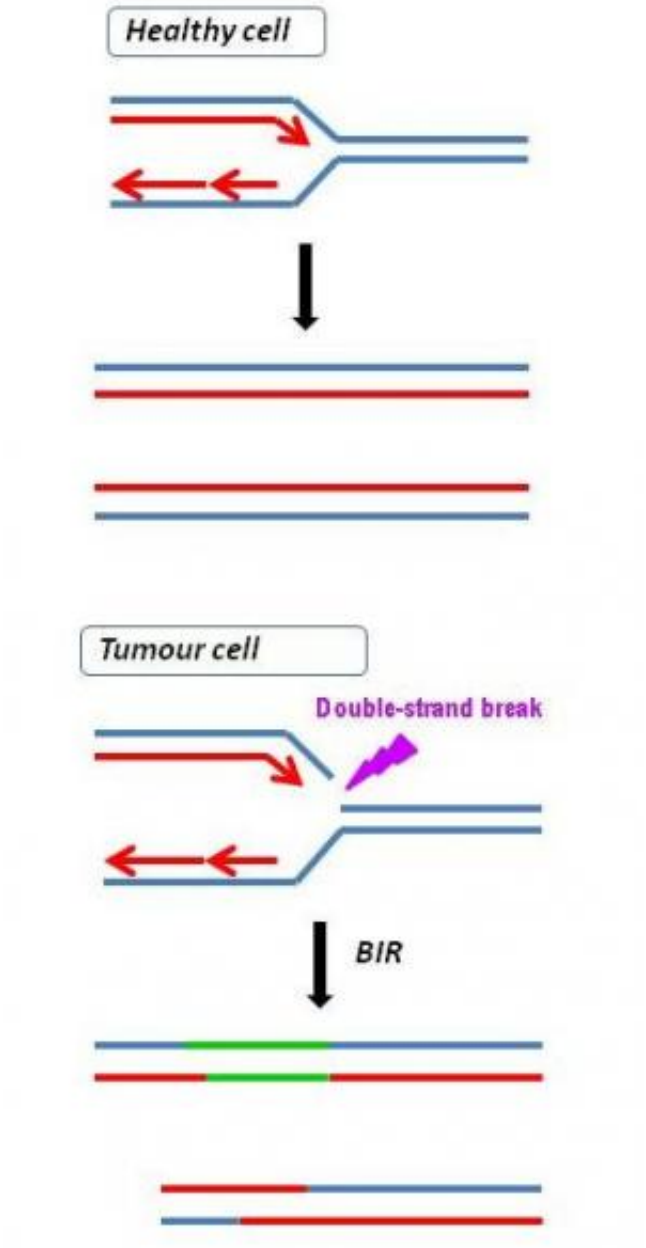

The parental DNA (blue) opens up to allow the replication of two new strands (red). The BIR repair process leads to the aberrant duplication of DNA fragments (green). Credit: © Thanos Halazonetis, UNIGE

Genomes must be replicated in two copies during cell division. This process occurs at structures called 'replication forks', which are equipped with enzymes and move along the separated DNA strands. In tumour cells, the replication forks are frequently damaged, giving rise to breaks in the double-stranded DNA. An international study led by Thanos Halazonetis, Professor at the Faculty of Sciences at the University of Geneva (Switzerland), has revealed how cancer cells repair the damaged replication forks in order to complete their division. The pathway used is known as 'break-induced replication' (BIR) and is common in cancer cells, but rare in healthy cells. The study described in the journal *Science* thus reveals a significant difference between these two types of cells, which its authors will attempt to exploit for therapeutic purposes.

For one of our cells to give birth to two daughter cells, it must first replicate its DNA which consists of around 6.4 billion pairs of nucleotides. The double-stranded DNA opens up like a zipper, producing a 'replication fork' upon which a group of enzymes move about. Present in different regions in the DNA, the forks move with the progression of the replication.

Cell proliferation is controlled in particular by specific genes known as proto-oncogenes. Their overexpression or mutation into oncogenes triggers an uncontrolled proliferation and promotes cancer development. 'In tumour cells, oncogenes induce a collapse or even a rupture of the replication forks. This causes the detachment of enzymatic replication complexes and a break of the double-stranded DNA', explains Thanos Halazonetis, Professor at the Department of Molecular Biology at the

University of Geneva.

The same mechanism in yeast and malignant cells

In collaboration with the universities of Helsinki (Finland), Duisburg-Essen (Germany), Brandeis (USA) and the Karolinska Institute (Sweden), the geneticist's team has determined how the damaged forks are repaired so that replication can resume. The researchers have analysed 690 genes involved in DNA metabolism. 'We have set up a library of molecules known as siRNAs which are capable of targeting genes individually by preventing them from being expressed', reports Lorenzo Costantino, post-doctoral fellow in the team and a main author of the article.

These genetic hooks have allowed the researchers to isolate several genes essential for the repair of damaged forks, including POLD3 and POLD4. These two genes encode proteins involved in DNA replication and repair. 'Thanks to these first hits, we were able to identify a different repair process known as 'break-induced replication' (BIR), which was known in yeast, but not in humans', notes Sotirios Sotiriou, doctoral researcher of the team and co-author.

Aberrant duplication of tumour DNA

The biologists have found that the BIR repair process, rarely employed in healthy cells, is very common in human tumour cells. Furthermore, the use of this intracellular repair pathway explains how abnormal duplications of portions of the genome observed in cancer cells occur. The genome's instability is in fact essential to tumour development as it allows for the accumulation of the prerequisite mutations. 'Different proteins, such as POLD3 and POLD4, are recruited for BIR. Our next objective is to identify all the other biochemical players involved in this

intracellular pathway in order to determine which ones could be a therapeutic target', explains Thanos Halazonetis.

More information: "Break-Induced Replication Repair of Damaged Forks Induces Genomic Duplications in Human Cells", *Science*, 2013.

Provided by University of Geneva