Tumour biology

Genome doubling causes double trouble

Yonatan Eliezer & Uri Ben-David

Human cancer cells often double their genome through an error in cell division, and this can lead to further genomic instability. A detailed analysis of the first cell cycle after genome doubling sheds light on this phenomenon. See p.146

Understanding how genomic abnormalities arise in cancer cells might offer new ways of developing treatments. On page 146, Gemble et al.[1] present an examination of the effects of genome doubling − an event that can lead to tumour formation.

Cancer evolves through the acquisition of genomic alterations, which perturb the normal regulation of cell proliferation and eventually enable tumour cells to divide and spread in an unregulated manner. Such alterations range in scale from small tweaks to the DNA sequence, called point mutations, to changes in the number or structure of whole chromosomes (termed aneuploidy), and even to doubling of the entire genetic content of the nucleus − a phenomenon called whole-genome doubling.

Whole-genome doubling (WGD) is a common event in various human cancers, being found in approximately 30% of tumours[2,3]. Cells that have two copies (maternal and paternal sequences) of the genome are called diploid, and WGD makes them tetraploid (they have four copies of the genome). Interestingly, tetraploid cells are prone to genomic instability and quickly acquire further genomic alterations[3–7].

Two main mechanisms have been proposed to explain the connection between WGD and genomic instability[8,9]. One is that if cells have four copies of each gene, this helps to cushion the cellular consequences of genetic alterations, enabling tetraploid cells to survive and tolerate such perturbations better than can cells with the usual number of copies of the genome[4–6,10]. The other proposed mechanism is that an excessively high number of chromosomes increases errors in the distribution of chromosomes between dividing cells, leading to DNA damage and chromosomal aberrations[11–14]. However, Gemble and colleagues demonstrate that tetraploid cells become genomically unstable even before division occurs.

The authors examined the immediate consequences of WGD, focusing on the stage of the cell cycle that occurs before division, called interphase. Interphase consists of three phases: G1, in which the cell grows and prepares for DNA replication; S, in which the cell doubles its genetic material through DNA
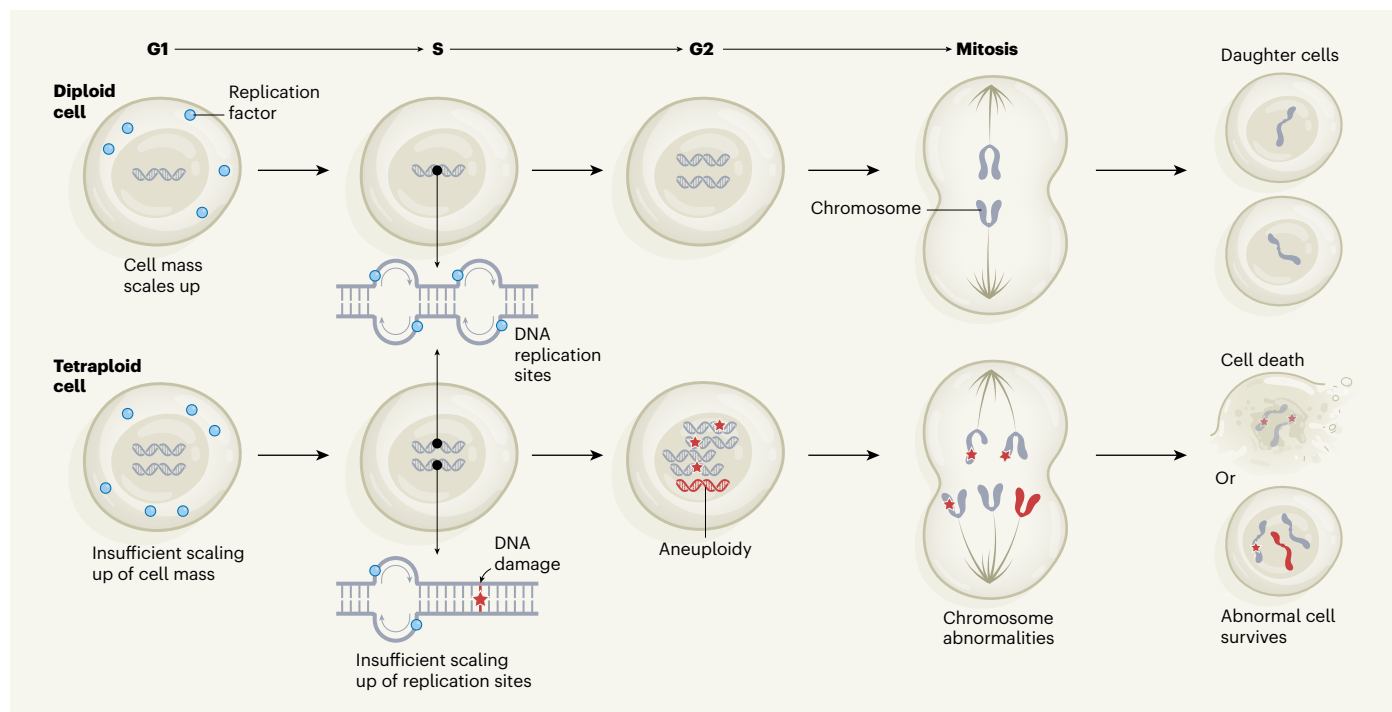

Figure 1 | The harmful effects of whole-genome doubling (WGD). Abnormal doubling of the entire DNA content of the nucleus is a hallmark of many tumours. Gemble et al.[1] induced WGD in human cells grown *in vitro* to assess the effects during the first cell cycle after WGD. A normal cell with two copies of the genome (termed diploid) scales up its cell mass during the G1 phase of the cell cycle, making components such as the replication factor proteins needed for DNA replication. Cells in which WGD was induced have four copies of the genome (termed tetraploid cells) and do not sufficiently scale up during G1. When tetraploid cells enter S phase and replicate DNA, they are unable to generate the expected number of DNA-replication sites, and this insufficiency causes a phenomenon called replication stress, which is associated with high levels of replication-dependent DNA damage. During the G2 phase of the cell cycle, as a consequence of their abnormal S phase, tetraploid cells exhibit other signs of genomic instability, such as the presence of extra DNA in the form of additional chromosomes. This change is termed aneuploidy. During mitosis, each diploid cell divides its chromosomes evenly between the daughter cells. However, tetraploid cells often fail to segregate their chromosomes properly during mitosis, which also drives aneuploidy. The DNA and chromosomal aberrations, along with the segregation problems, can result in cell death. Or if the abnormal cells survive, the genomic havoc might ultimately result in tumour formation.

replication; and G2, in which the cell prepares for division. The cell cycle ends in a stage called mitosis, when division occurs and the genomic content is separated into two daughter cells.

Gemble and colleagues induced WGD in human cells grown *in vitro* and monitored interphase (Fig. 1). The authors triggered WGD using various approaches, representing three major mechanisms by which WGD is known to occur. Focusing on the first interphase to occur after WGD offered a way of avoiding a common outcome of doubling — cell-cycle arrest, which often prevents analysis of newly formed tetraploid cells[4,15].

Using time-lapse microscopy of live cells, the authors observed several differences between tetraploid and diploid cells. First, high levels of DNA damage occurred during the first interphase after the induction of WGD, regardless of the genome-doubling mechanism applied. This result was consistent for several types of human cell and also when cells were grown in 3D cultures. Second, the high degree of DNA damage was mostly associated with S phase and the process of DNA replication. This was shown by chemically blocking progression of the cell cycle and assessing DNA damage at each step. Moreover, treatment of tetraploid cells with drugs that inhibited DNA replication caused a marked decrease in signs of DNA damage. Third, the authors carried out single-cell DNA sequencing, and observed abnormal chromosome compositions (called karyotypes) in tetraploid cells after a single S phase, even before mitosis began. These findings indicate that genome doubling results in DNA-replication-dependent DNA damage.

A plausible explanation for how such damage arises is replication stress, a condition in which the cell experiences difficulties during S phase, and replication stalls. When the authors chemically induced such stress in diploid cells, levels of DNA damage were generated that were comparable to those observed after genome doubling. Indeed, proteins associated with DNA damage co-localized in the nuclei of tetraploid cells with proteins associated with active DNA replication, highlighting a link between the two processes. Using a method to visualize and quantify DNA replication, called DNA combing, the authors report that tetraploid cells failed to produce enough replication sites (Fig. 1) for doubling of the genomic content, and that this led to rapidly progressing, unstable replication.

A common cause of replication-stress-dependent DNA damage is the depletion of nucleotides, the building blocks of DNA[16]. However, addition of these components did not reduce the damage, eliminating this as a possible explanation. Gemble *et al.* observed that the cell mass of tetraploid cells failed to increase as expected during the first

G1 after WGD. Growth of normal cells during G1 prepares them for DNA replication through the accumulation of key proteins needed to regulate S phase. In tetraploid cells, however, there was a shortfall of these factors.

The authors report that DNA damage was reduced when tetraploid cells were given more time to prepare for S phase through a manipulation that lengthened G1. Such damage was also reduced by generating higher than normal levels of the protein E2F1, a transcription factor that activates S-phase-associated genes[17]. Gemble *et al.* conclude that tetraploid cells transition prematurely from G1 to S without

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adequately scaling up their protein mass and without sufficiently stockpiling factors needed for DNA replication. This results in substantial DNA damage when replication is attempted. Of note, G1 lengthening and E2F1 overexpression did not fully block DNA damage, indicating that other processes are also involved.

The authors' work demonstrates that the genomic and chromosomal instability that characterizes tetraploid cells can occur even before a cell divides. Crucially, most of the authors' observations held true across cell types and regardless of the mechanism used to induce WGD. In addition, the key findings were recapitulated *in vivo*, using neuroblast cells from *Drosophila* flies. This raises the question of whether the findings have potential relevance to human cancer.

Cancer is mainly attributed to the continuous evolution of cells that accumulate genetic changes. Nevertheless, it can also progress through evolutionary bursts triggered by major catastrophic events, such as a chromosome 'shattering' process called chromothripsis[18]. If the mechanism Gemble and colleagues report applies to human cells *in vivo*, this suggests that WGD might contribute to tumour evolution not only by driving gradual genomic evolution during the course of cell divisions[4,11,13], but also by inducing an abrupt cellular catastrophe. If so, one might expect to identify a unique genomic 'scar' in tumours that arose in this manner.

The authors observed increased expression of particular genes associated with the response to DNA damage in tetraploid (compared with diploid) human tumours, although this might be the consequence of

either mitosis- or interphase-induced DNA damage. Future studies should use animal models and analyse clinical genomic data to assess whether genomic scars unique to this interphase phenomenon can be found, and to determine whether there are any differences between DNA damage induced during S phase and that induced during subsequent mitoses.

It is worth remembering that Gemble and colleagues' results were obtained in cells cultured under non-physiological conditions, which did not have an opportunity for cellular adaptation. As many studies[4,15] have reported, and the authors also found, genome doubling often results in eventual cell-cycle arrest and cell death, suggesting that cells that have undergone DNA damage in interphase might be more likely to die than to become cancerous. Previous studies[14,19] using stable, dividing tetraploid cells report the scaling-up of DNA and protein content, which suggests that some tetraploid cells overcome an initial S-phase crisis or do not experience such a crisis at all. Future research will surely attempt to uncover the molecular mechanisms that enable cells to tolerate genome doubling, to bypass or mitigate its immediate detrimental consequences, and to keep on dividing. In the meantime, Gemble and colleagues' work makes it clear that genome doubling jeopardizes genomic integrity in more ways than were previously appreciated.

Yonatan Eliezer and **Uri Ben-David** are in the Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel.
e-mail: ubendavid@tauex.tau.ac.il

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