TUMOUR BIOLOGY

Watching cancer cells evolve

Chromosomal abnormalities are a hallmark of many types of human cancer, but it has been difficult to observe such changes in living cells and to study how they arise. Progress is now being made on this front.

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The genomes of cancer cells are littered with mutations (errors in individual nucleotides), some of which might contribute to growth of the cancer by activating tumour-promoting genes called oncogenes, or by switching off genes belonging to a class known as tumour suppressors, which fight cancer. Yet, arguably even more important are the genomic abnormalities that occur in tumour cells on a much larger scale. For example, such a cell might contain anomalous numbers of entire chromosomes (a situation termed aneuploidy). As the tumour evolves, chromosomal abnormalities can vary between neighbouring cancer cells. This suggests that chromosomal changes can occur by repeated chromosomal 'shuffling' during each cell division, resulting in a high rate of genomic change, termed chromosomal instability.

Technical difficulties in analysing cells freshly isolated from tumours have previously prevented researchers from determining whether there is ongoing chromosomal instability in tumours. Writing in *Nature Genetics*, Bolhaqueiro *et al.*[1] report such data, revealing ongoing chromosomal instability in human colorectal cancer cells.

It has been known for more than 20 years that cells in most colorectal cancers vary in the number of their chromosomes[2], and this has been attributed to chromosomal instability. Nearly 90% of solid tumours have some degree of chromosomal instability[3]. This continuous alteration of a tumour-cell genome is thought to aid the cells' rapid adaptation to the effects of anticancer therapies. A high level of genomic alteration is often correlated with poor prognosis for people who have cancer[4,5]. There is therefore great interest in gaining a clear picture of chromosomal instability, for example determining the mechanisms responsible, the rate at which such changes occur and how it evolves in a single tumour.

However, given that chromosomal instability is a dynamic process, it cannot be adequately assessed solely from the isolated DNA-profiling 'snapshots' of a tumour genome that are typically available. So far, much of the backbone of laboratory work illuminating the rates and mechanisms involved[6–8] has been based on experiments in which cells that have been removed from tumours are grown in plastic dishes, usually as a 2D monolayer, and such cells are often maintained in culture for many years.

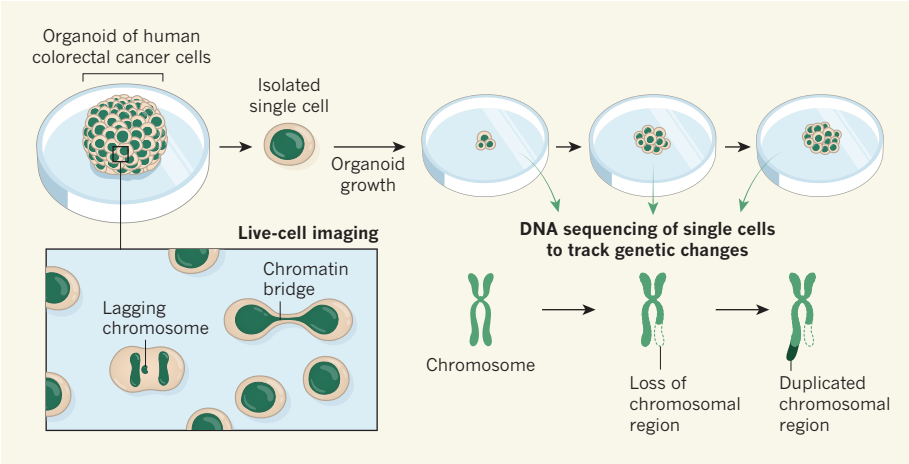

Figure 1 | Tracking chromosomal changes in human colorectal cancer. The emergence of chromosomal abnormalities in colorectal cancer is associated with poor prognosis, and understanding how such changes occur in living cells might reveal targets for drug development. Bolhaqueiro *et al.*[1] studied samples of human colorectal cancer cells that had been grown *in vitro* as a 3D clump of cells called an organoid. Engineering the cells to express a fluorescent DNA-binding protein enabled the authors to use microscopy to image the genetic material (green) as the cancer cells divided. This provided insights into the cell-division errors that occurred, such as a chromosome becoming separated from the rest of the chromosomes as a cell divides, termed a lagging chromosome (inset). The most common chromosomal abnormalities observed were structures called chromatin bridges, which are regions of DNA formed from chromosomes that have fused. The authors also conducted experiments in which they isolated a single cell from the organoid, and used it to grow another organoid. By periodically conducting single-cell DNA sequencing of this growing organoid, chromosomal alterations, such as deletions or duplications of chromosomal regions, could be tracked over time.

Just how representative these 2D model systems are of tumours growing in their native environment remains unclear. Cell-culture systems that can enable 3D growth, such as using clumps of cells called organoids, have been pioneered as a way of mirroring tumour environments more accurately than is possible using 2D systems[6,9]. Indeed, when the normal tissue architecture surrounding non-cancer cells is lost by culturing the cells in a 2D monolayer, this can suffice to promote error-prone cell division[10]. However, abnormal cell divisions do not occur if the cells are grown as organoids[10].

To study chromosomal instability in living cells, Bolhaqueiro *et al.* isolated samples of human colorectal tumour cells, and cultured the cells directly as organoids. By genetically engineering the cells to express a fluorescent DNA-binding protein, the authors could carry out live imaging to assess chromosomal behaviour during cell division (Fig. 1). They also performed periodic single-cell DNA sequencing to track changes in chromosomal number and structure (the order of DNA sequences on chromosomes) over time.

The authors found that dividing cancer cells frequently made errors in the partitioning of chromosomes between daughter cells, confirming results previously obtained using 2D systems[7,8]. They did not observe such errors in organoids made from healthy colon tissue, indicating that the experimental system itself does not trigger such errors. These partitioning defects were indicative of an ongoing process of chromosomal instability. The authors tested whether this was the case by studying organoids derived from a single tumour cell that was monitored as the organoid grew

and evolved (Fig. 1). Carrying out single-cell DNA sequencing at different times during this experiment confirmed that there was ongoing chromosomal instability.

It has previously been difficult to assess the mechanisms that generate this instability in a native environment. Bolhaqueiro and colleagues' organoid model is as close a representation of that as is so far possible. The authors noted that most of the chromosome-partitioning errors arose from the formation of chromatin bridges, in which a chromosome, or part of one, is stretched between the two separating masses of cellular DNA at the final stage of cell division. Such errors are indicative of structural changes during chromosome replication that occur before cell division, and have been observed in 2D monolayers of colorectal cancer cells[8].

There is much debate about the cause of chromosomal instability in cancer. Chromatin bridges could arise as a result of other defects in cell-division processes, and further in-depth analyses using organoid models to investigate the specific nature of such chromosome-partitioning defects and any other abnormal cell-division processes might shed light on this.

A subset of human colorectal cancers do not have the usual hallmarks of chromosomal instability, and although they have a high level of nucleotide mutations (termed microsatellite instability), they have normal or almost normal chromosome numbers, and there is little chromosomal difference between cells. Bolhaqueiro *et al.* made the surprising discovery that some organoids of this subtype of colorectal cancer also have appreciable rates of chromosomal errors during cell division. DNA sequencing of single cells obtained from clinical samples of tumour tissues by Bolhaqueiro and colleagues revealed that chromosomal instability can occur along with microsatellite instability in the same tumour. The low level of chromosomal instability in these tumours probably hindered its detection in earlier bulk DNA-sequencing methods that did not use a single-cell sequencing approach.

Bolhaqueiro *et al.* also noted that, for each individual organoid, there was only a weak correlation between the number of times that cells made errors in chromosomal partitioning during cell division and the level of variation in chromosome numbers between cells in the organoid. This discrepancy would make sense if there were differences in how some organoids respond to chromosomal-partitioning errors. Live-cell imaging indeed revealed that organoids with a high variability in chromosome number were better able to withstand errors during cell division than were organoids that had low chromosome-number variability. If such errors occurred in organoids that had low variability in chromosome number, then cell division often stopped or the cells died.

These results suggest that chromosome-number variability in tumour cells is the product of the rate of development of chromosomal instability and the ability of cells of a given tumour type to tolerate further changes in chromosome number or structure[11]. The factors that govern whether or not a tumour will tolerate aneuploidy remain mostly unknown. A better understanding of this would probably reveal potential targets for the development of new therapies.

As the use of organoid model systems becomes more widespread, experiments that can directly compare the results obtained in 3D and 2D cultures would help to ensure that the appropriate culture systems are chosen for the specific research question and experimental approach involved. This would allow more-direct comparisons between the results from different groups. With regard to Bolhaqueiro and colleagues' work, it would be interesting to know whether the rate of chromosomal instability and aneuploidy observed would have been different had 2D growth systems been used.

It would also be helpful to know how representative previous work in 2D monolayer systems is of what happens *in vivo*, and which questions might need to be re-evaluated using organoids. For example, although a normal 3D tissue architecture might be essential for preventing chromosomal instability during cell division in non-cancer cells, it is possible that, if cancer cells have already lost the normal checkpoints to combat faulty cell-division processes, such cells will exhibit similar rates and mechanisms of chromosomal instability regardless of whether they are grown in 2D or 3D systems. If so, organoid studies might be needed only to confirm those carried out in 2D systems, rather than being essential.

Finally, aneuploidy is emerging as a possible early event driving tumour evolution[12,13], but information about the frequency and types of chromosomal instability during the initial stages of tumour evolution is extremely limited. If organoids could be used to examine chromosomal instability during these preliminary stages, or to study abnormal cells (called precursor lesions) that might be on a pathway to forming cancer, this would undoubtedly yield many fascinating insights. ■

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ATMOSPHERIC CHEMISTRY

Ozone mystery laid to rest

Measurements of atmospheric ozone levels taken during the nineteenth century cast doubt on the computational models used today to simulate the atmosphere. An independent proxy of past ozone levels offers reassurance. SEE LETTER P.224

MATHEW EVANS

Before the 1950s, direct observations of the composition of the atmosphere were extremely limited. Air trapped in pockets of snow and ice has allowed the observational record for some gases to be extended back hundreds of years. But certain gases that affect climate and air quality, such as ozone (O_3), are not stable in ice or snow, limiting their records to the past few decades. On page 224, Yeung *et al.*[1] report that isotopic observations of oxygen (O_2) molecules trapped in polar snow and ice can provide a new constraint on estimates of ozone levels in the troposphere (the lowest 12 kilometres of the atmosphere) over the past 150 years. This greatly extends our knowledge of the concentration of this key atmospheric gas, and might finally address a problem that has worried atmospheric chemists for decades.

Advances in atmospheric science are often made by taking advantage of the 'experiments of opportunity' that occur as a result of natural and human-driven changes to the atmosphere. It is therefore crucial to make long-term measurements of the atmosphere. The modern era for such measurements began in the late 1950s, with observations of carbon dioxide levels[2] from the Mauna Loa Observatory in