Cancer genetics

Genomes captured during tumour spread

Jillian F. Wise & Michael S. Lawrence

A better understanding of the genetic changes that enable cancers to spread is crucial. A comprehensive study of whole-genome sequences from metastatic cancer will help researchers to achieve this goal. See p.210

The major cause of cancer-related deaths is the spread of cancer cells from their primary site to other parts of the body[1]. This spreading process, known as metastasis, typically involves cellular stressors and environmental shocks that induce dramatic changes in cancer cells. One such change is a fierce resistance to current therapies, which means that new ways to combat metastatic disease are urgently needed. On page 210, Priestley *et al.*[2] use whole-genome sequencing (WGS) to illuminate the genomic changes that underpin metastasis in 22 types of solid tumour. Although previous studies[3,4] have unearthed some hints of such changes, this is perhaps the first pan-cancer metastasis study of its size to exploit the power of WGS.

Priestley *et al.* characterized 2,520 samples of metastatic tumours from people with cancer (Fig. 1). In each case, they also analysed a sample of non-cancerous blood cells from the same person. Using WGS, the authors produced a rich catalogue of the genetic mutations found in each metastasis. This catalogue complements existing inventories from both metastasis-sequencing studies and genomic databases of primary tumours, and offers several interesting insights. For example, the authors reveal frequent mutations in the gene *MLK4*; this is consistent with a previous study that connected an increased number of copies of *MLK4* with metastasis[5].

Most of the authors' findings confirm previous work on metastatic cancers[3,4]. For instance, other studies did not find recurrent cancer-causing mutations that were specific to metastatic tumours (that is, absent in the primary tumour) and that thus might have triggered metastasis. This has led to speculation that, at least in solid tumours, metastasis-specific mutations are not the major cause of cancer spread[1]. Priestley *et al.* also found limited evidence of such mutations.

The researchers analysed not only single-nucleotide (point) mutations, but also large structural variations, including the deletion of DNA sequences and translocations of DNA from one chromosomal region to another. Structural variations are difficult to detect using sequencing techniques that cover small portions of the genome – sequencing of only protein-coding regions, for instance, or of even smaller targeted sequences. These techniques are used more frequently than WGS in clinical studies because of their affordability. Documentation of large structural variants is therefore a valuable feature of Priestley and colleagues' WGS study.

In particular, the report reveals pervasive whole-genome doubling (WGD), in which the entire chromosome inventory is copied. Priestley *et al.* find WGD in up to 80% of cases in certain types of metastatic cancer, whereas the phenomenon has been reported in only about 30% of primary tumours[6]. Linked to chromosomal instability, WGD can confer multidrug resistance to chemotherapy. Furthermore, it might provide a buffer for cancer cells against the detrimental effects on fitness caused by genomic instability, such as damaging mutations and losses of chromosomal segments[7].

Although Priestley and colleagues present a landmark study, future efforts could benefit from researchers also sequencing each person's primary tumour. Doing this would have allowed Priestley *et al.* to generate a detailed reconstruction of how each cancer's genome evolved along the route to metastasis. To compensate for this limitation, the authors leveraged a large WGS study of primary tumours (the International Cancer Genome Consortium's pan-cancer analysis of whole genomes[8]). The researchers compared point mutations and small insertions and deletions between the two studies. These analyses largely confirmed a previous report of high genomic concordance between primary and metastatic tumours[9]. However, the comparison also revealed that the ten most commonly mutated cancer-causing genes in primary tumours are even more frequently mutated in metastatic tumours. Furthermore, larger DNA aberrations such as structural variations and WGD are significantly more common in metastases in most cancer types. These findings indicate that a hallmark of metastatic progression is ongoing and accelerating genomic instability.

Another caveat concerning this study, acknowledged by the authors, involves the use of fine-needle biopsies as the major sample-collection method. These biopsies gather cells from only a tiny subregion of a

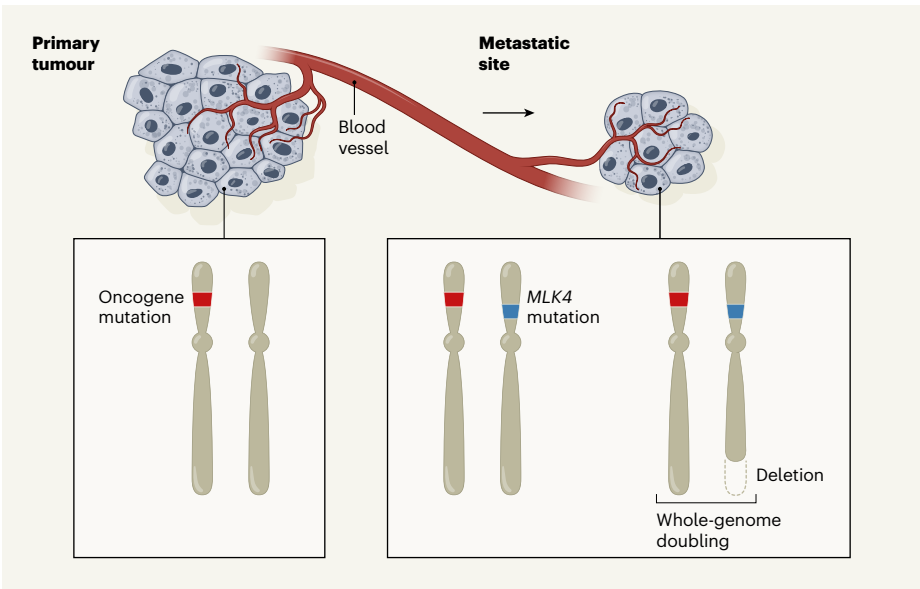

Figure 1 | Characteristics common across metastatic cancers. Cells in a primary tumour typically harbour cancer-causing mutations (oncogenes). As the cancer evolves, it acquires further mutations that enable it to spread to other sites in the body through the blood – a process called metastasis. Priestley *et al.*[2] sequenced the entire genomes of 2,520 metastatic tumours, across 22 cancer types. They find frequent mutations in the gene *MLK4*. They also report widespread structural variations, such as whole-genome doubling (which they find to be especially common) and deletions of large chromosomal regions.

metastatic site. The authors report that, on average, more than about 93% of mutations detected in a given sample were present in every cell of that sample. This is in stark contrast to previous studies[10], which have reported much higher levels of variation. The extreme homogeneity observed by Priestley *et al.* could, in principle, reflect the fact that only a few founding cancer cells colonized each metastasis, but might instead reflect the limited regional sampling achieved by the fine-needle biopsy method.

Future clinical studies of metastasis are likely to consider liquid biopsies as an alternative collection method. Liquid biopsies involve collecting samples of a person's blood and applying specialized laboratory techniques to isolate cancer-derived components, such as circulating tumour cells, circulating tumour DNA and released subcellular vesicles. This approach is less invasive than fine-needle or surgical biopsies. It also offers other advantages, including the ability to collect cells simultaneously from all metastatic cancer sites in the body (instead of just one), and to repeat sampling at multiple times during treatment, thereby providing dynamic temporal information about a cancer and its response to therapy. Liquid biopsies also enable researchers to document metastatic evolution at the DNA, RNA and protein levels in parallel[11,12].

Ultimately, the true value of any research comes from improvements to treatment. To maximize the potential for clinical impact, Priestley and colleagues' data set is open-access. The authors have already accumulated more than 80 collaborative requests to investigate topics ranging from the possible presence of viral genetic material in the samples to the relationship between the sequences and patient drug responses (go.nature.com/2ommmn2). The data set is also being used to investigate whether any mutational variants involved in driving metastasis lie in regulatory DNA regions, and to enable efforts to deduce the anatomical origin of metastatic cancers diagnosed without a known primary-tumour site. Indeed, it is already powering exploration of these questions. The publicly available repositories are also being used in a Drug Rediscovery protocol[13], in which patients with metastases who have exhausted standard therapies are matched with promising off-label treatments (anticancer medicines that have not been specifically approved for use against the person's type of cancer) on the basis of results from WGS.

Obtaining metastatic biopsies is not without risks to the patient, such as bleeding and infection. This is partly why sample collection has been so limited until now. Those who donated samples to this study have provided researchers with a valuable gift. It is hoped that the database will, in turn, provide the new insights and therapeutic strategies that are so urgently needed.

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Experimental physics

Progress on the proton-radius puzzle

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Atomic physicists and nuclear physicists have each made a refined measurement of the radius of the proton. Both values agree with a hotly debated result obtained by spectroscopy of an exotic form of hydrogen called muonic hydrogen. **See p.147**

The proton, discovered 100 years ago[1], is an essential building block of visible matter. The nucleus of a hydrogen atom consists of a single proton, making this atom a suitable platform for determining the proton's intrinsic properties. One such property is the proton charge radius, which corresponds to the spatial extent of the distribution of the proton's charge. In 2010, a highly accurate measurement of the proton radius was made using spectroscopy of muonic hydrogen — an exotic form of hydrogen in which the electron is replaced by a heavier version called a muon[2]. However, the value obtained was almost 4% smaller than the previously accepted one[3]. Bezginov *et al.*[4], writing in *Science*, and Xiong *et al.*[5], on page 147, report experiments that could represent a decisive step towards solving this proton-radius puzzle.

Atomic physicists determine the proton radius by measuring the energy difference between two electronic states of a hydrogen atom using spectroscopy. According to quantum mechanics, there is a non-zero probability that the electron will be found inside the proton if the electron is in a rotationless state (an *S* state). When inside, the electron is less strongly influenced by the proton's electric charge than it would otherwise be. This effect slightly weakens the binding of the electron and proton, and causes a tiny shift in the energy of the *S* state with respect to other states. The high precision achieved both by experiments and by the theory of quantum electrodynamics allows this energy shift and, in turn, the proton radius, to be extracted from measurements.

A muon is about 200 times heavier than an electron. As a result, there is a much higher probability that the muon in a muonic-hydrogen atom will be found inside the proton than would the electron in an ordinary hydrogen atom. Consequently, the associated energy shift is about 8 million (200^3) times larger for muonic hydrogen than for regular hydrogen[6]. Muonic hydrogen is therefore a highly sensitive probe of the proton radius.

Bezginov and colleagues' work concerns the Lamb shift of ordinary hydrogen — the energy difference between the $2S$ and $2P$ excited states. This shift was investigated previously in muonic hydrogen[2,7]. To measure the Lamb shift, the authors developed an experimental method[8] that derives from a technique known as Ramsey interferometry, which is used in atomic clocks.

This experimental method has many technical advantages over other approaches with regard to eliminating systematic uncertainties, filtering environmental noise, and simplicity in the shape of the spectral signal. A key feature of the set-up is the ability to measure a full spectrum in only a few hours. This allowed Bezginov *et al.* to carry out a meticulous study of systematic uncertainties and to extract a precise value for the proton radius: 0.833 ± 0.010 femtometres (1 fm is 10^{-15} metres).