indicates genes that are probably poised for a rapid response when infection occurs. The approach highlighted a group of genes encoding a substantial number of immune-associated proteins, and examples of these were most evident in structural cells from the skin, liver and spleen. These genes are worthy of further study that focuses on how the structural cells that express them respond to infection and protect the organ that is their home.

The authors confirmed that they had indeed identified genes poised for a role in an immune response by infecting mice with lymphocytic choriomeningitis virus (LCMV) and then monitoring gene expression by RNA sequencing of structural cells. LCMV is a well-studied virus that affects most organs, and this allowed Krausgruber and colleagues to distinguish organ-specific from global defence responses. Eight days after infection, up to 57.9% of the genes of unrealized potential had been activated in structural cells, with notably high responses in fibroblasts and endothelial cells in the liver, spleen, lungs and large intestine.

Furthermore, the authors found that an antiviral response was evident in these gene-expression profiles. When infected and non-infected animals were compared, the infected animals had higher levels of expression of transcription factors and immune-associated signalling proteins called cytokines that are involved in pathways associated with expression of the antiviral protein interferon. In response to the viral infection, structural cells also expressed small proteins called chemokines that attract immune cells. This was a surprise, because chemokine secretion has been mainly associated with immune cells. The authors propose that their predicted interaction network between immune cells and structural cells is altered on LCMV infection, and suggest that, on infection, structural cells in various organs increase interactions with immune cells such as monocytes, macrophages and B cells.

To dissect the effects of signalling in response to LCMV infection, the authors injected individual cytokines, of types detected in the antiviral response, into the bloodstream of mice that did not have an LCMV infection. Krausgruber *et al*. then sequenced the RNA in structural cells from the organs with the greatest previously observed response to LCMV. They found that gene-expression changes were more evident in fibroblasts and endothelial cells than in epithelial cells. Dissecting the gene-expression response to each cytokine revealed the portion of the antiviral program that it controls. Among other interactions, this revealed that the cytokines IL-6 and IFN-γ, possibly produced *in vivo* by immune cells, are responsible for eliciting much of the antiviral response of spleen endothelial cells by driving the expression of genes with unrealized potential.

Although gene-expression programs involved in the immune response have been reported previously for some structural cells, Krausgruber and colleagues' work underscores these cells' decisive role in coordinating organ-specific and organism-wide immune responses. It also indicates how functionally relevant candidate genes can be pinpointed using a combination of cell-communication networks and analysis of chromatin-mediated regulation. One of the ultimate goals of this research field could be to develop cell-type-targeted therapies that modulate immune responses. This could greatly benefit cancer research, for example, because cancer-associated fibroblasts have a role in promoting tumour progression[5].

Future studies will probably focus on the defence responses of other types and subtypes of human cells in studies linked to the Human Cell Atlas initiative[6], which is generating detailed molecular profiles for all human cells to fully describe cell-type diversity. Single-cell approaches could assist in profiling the RNA transcripts in all cell types and states of entire organs, in steady-state and post-stimulus scenarios. The use of a new method called spatial transcriptomics (which monitors gene expression in intact tissue sections rather than in dissociated cells), together with information about chromatin status, could disentangle the entire cellular chain of events, from the detection of infection to the defence response and immune-cell recruitment, and then finally to the removal of the infectious agent. By profiling structural cells in different mouse organs, Krausgruber *et al*. have unlocked a trove of knowledge about antiviral defences, which might be relevant to other species and facilitate new ways to target human diseases.

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Cancer evolution

Strands of evidence

Trevor A. Graham & Sarah E. McClelland

DNA damage can cause mutations due to failure of DNA repair and errors during DNA replication. Tracking the strand of the DNA double helix on which damage occurs has shed light on processes that affect tumour evolution. **See p.265**

How a cancer evolves and how mutations are generated are highly intertwined processes, and both are nearly impossible to observe directly. Instead, we are usually restricted to making inferences about them using data from a single snapshot in time after a cancer has formed. Aitken *et al*.[1] show on page 265 that, for a cell that has undergone DNA damage, such a snapshot provides remarkably rich information when the two DNA strands that form the double helix are considered independently.

DNA resembles a ladder, with the two 'side rails' often called, respectively, the Watson and Crick strands. These are fused together by 'rungs' of two complementary nucleotide base pairs: either cytosine (C) paired with guanine (G) or adenine (A) paired with thymine (T). When a cell divides, each daughter cell inherits either the Watson or Crick strand from the parent; this provides a template from which the other, complementary strand is replicated. Damage to a base can trigger a repair process, but if repair is not swift enough, the damaged base might be mispaired with an incorrect base during DNA replication. At the next round of cell division, when a daughter cell with such a mispaired base prepares to divide, the base complementary to the mispaired base will be added to the newly synthesized strand. This leads to a double-stranded mutation at the base pair corresponding to the original damaged base (Fig. 1).

Standard practice for genome sequencing is to consider mutations without paying attention to which of the strands received the original damage. However, when a chemical change occurs that damages a base, creating a site referred to as a lesion, this lesion is on only one of the two DNA strands of the affected base pair. Aitken and colleagues had the insight to see that, because

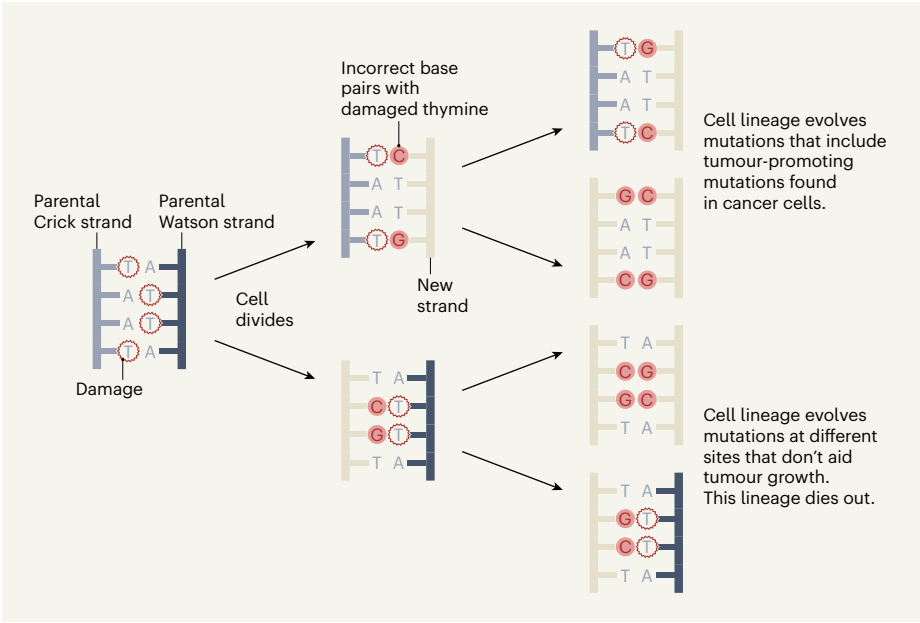

Figure 1 | Tracking the connection between damage to individual DNA strands and tumour mutations. Aitken *et al.*[1] analysed mutation patterns in the cells of mice that received a carcinogenic molecule called diethylnitrosamine, which damages thymine (T) nucleotide bases. Damage is indicated by red circles. The cell originally exposed to diethylnitrosamine has two DNA strands, named here as the parental Crick and Watson strands. The correct pairing of bases is either adenine (A) paired with T or guanine (G) paired with cytosine (C). When the original cell divides, an incorrect base (shown in pink) can mispair with a damaged T base. Then, as those two daughter cells divide, the incorrect base will pair with the complementary matching base (for example, a mispaired C pairs with a G on the newly made strand), which results in both DNA strands having a mutation at that particular base pair. The original cell division generates two cell lineages that have mutations arising from damage to either the parental Crick or parental Watson strand, respectively. Each of these lineages has mutations at distinct base-pair locations. As the cells continue to divide, mispairing opposite unrepaired damaged T bases continues, producing different mutations at the same base-pair position and generating genetic diversity. Only cell lineages with mutations that aid tumour growth will be found in a tumour that the mouse subsequently develops. This example shows one possible scenario, in which mutations arising as a consequence of damage only to the parental Crick strand contribute to cancer growth.

the 'parental' Watson and Crick strands of an original cell that underwent DNA damage are separated into different daughter cells, when the cell divides, two cell lineages can be tracked individually by following the unique pattern of mutations that lesions on each of the parental strands generates.

To induce DNA lesions, Aitken and colleagues gave mice a large dose of the carcinogenic molecule diethylnitrosamine. This treatment predominantly caused DNA lesions at T bases in liver cells, ultimately leading to tumour growth. When the authors examined the pattern of diethylnitrosamine-induced mutations along the genome of each tumour, they found long stretches of the genome that, compared with the original, unmutated genome, were highly enriched for mutations in which T was mutated to any other base (N). These mutations were derived from T lesions on the DNA strand (let's call it the Crick strand) that was inherited by the daughter cell, and its cellular descendants, that went on to form the tumour. Lesions on either strand can generate mutations, but it might be the case that lesions on only one of the parental strands generates tumour-promoting mutations and hence only one of the two daughter lineages forms a tumour. In the example shown in Figure 1, the lesions on T bases on the corresponding Watson parental strand received by the other daughter cell did not lead to the formation of tumour-promoting mutations, and this cellular lineage therefore did not contribute to the tumour.

The authors realized that the pattern of base-pair locations that had T-to-N mutations enabled them to pinpoint the individual Watson or Crick strand that had served as the template strand for the first cell in the tumour. This template strand carried diethylnitrosamine-induced lesions mainly at T bases, allowing the fate of each strand to be tracked individually through subsequent cell divisions (Fig. 1). These individual 'strands of evidence' provide remarkable information about the process of mutation and tumour evolution.

In gene expression, DNA is transcribed to produce RNA, and DNA lesions can be repaired by a process called transcription-coupled repair[2]. Aitken and colleagues observed that transcription-coupled repair occurred preferentially on the strand being transcribed, as opposed to the complementary strand, and that higher levels of transcription were associated with an increased frequency of repair.

The authors found that failure to repair a DNA lesion over successive cell cycles, an interesting observation in itself, provided an unexpected source of genetic diversity. Each round of DNA replication on a lesion-containing strand could lead to the incorporation of a different 'wrong', mispaired base opposite the lesion site in the newly synthesized strand. If this happened, it caused further, distinct mutations at the same genomic position, generating cells in the tumour each with different mutations of the same base pair.

Observing recurrent mutations at the same genomic site could be taken as evidence of convergent evolution, in which multiple individual mutational events at that base-pair site are all positively selected for during tumour growth. Instead, Aitken and colleagues' findings indicate that recurrent mutations could result from lesion-bearing DNA strands being used as templates for DNA replication over multiple rounds of cell division.

Intriguingly, strand tracing also provides a window on the selection of mutations associated with cancer. When a cell divides, a daughter cell should inherit, at random, either DNA strand. However, when the authors tracked the prevalence of sequences corresponding to inheritance of the parental Watson or Crick strand of a particular chromosome, they noticed that the tumours contained one of these two strands more often than would be expected by chance.

The authors' explanation for such preferential strand retention is that it occurred because the retained strand contained a diethylnitrosamine-induced mutation in a gene that is important for tumour growth. Aitken *et al.* identified three potential tumour-promoting genes in this way, all of which are known to be crucial for the growth of liver tumours. Strand-by-strand analysis might be an unexpectedly useful tool for probing the tumour-promoting contribution of non-protein-coding regions of the genome, because selection can be detected without needing to know the background mutation rate – the problem of determining this rate has posed a challenge for methods previously used to study these regions[3].

It might be expected that because there is strand-biased prevalence of mutations corresponding to diethylnitrosamine-induced lesions, a chromosome should be enriched for T-to-N mutations along the entire length of its DNA strand. Instead, the authors found that, for a single chromosome, the enrichment of such mutations sometimes switched over to the other strand (and could be observed as A-to-N mutations). They propose that this provides evidence of sites of a DNA-repair process called homologous recombination, in which DNA strands from a chromosome exchange

with identical DNA sequences in a cell that is gearing up to divide, during an event called sister-chromatid exchange.

Sister-chromatid exchange is usually an elusive process to monitor because it involves, in theory, an exchange between two identical DNA sequences. However, because Aitken and colleagues could track individual strands through mutation patterns, they could detect evidence of such events. Interestingly, a higher frequency of these exchange events tracked with a higher diethylnitrosamine-induced mutation burden, suggesting that diethylnitrosamine-induced damage to DNA might prompt sister-chromatid exchange[4]. Aitken *et al.* report that presumptive exchange events tended to occur in regions of the genome that were associated with lower-than-average gene expression and later replication, compared with other regions, during the cell cycle. Interestingly, these features are hallmarks of 'fragile sites' — genomic regions susceptible to damage during DNA replication that are known to be prone to sister-chromatid exchange[5,6].

The most tantalizing question raised by this study is whether the DNA strand (the original Watson or Crick strand) in which damage first occurred can be tracked in human cancers. People are usually exposed to mutation-causing agents, such as those in cigarette smoke, for long periods of time, making it probable that DNA lesions are continually being induced. Consequently, the mutational signal arising from an individual DNA strand would probably be obscured. However, when Aitken and colleagues assessed data already available from human cancers, they found that, in rare cases of sudden, acute exposure to a mutagenic agent, most clearly observed for aristolochic acid exposure (which leads to liver, kidney and bile-duct cancer), mutational signals could be traced back to identify the strand originally damaged.

Chemotherapy also provides an example of acute exposure to mutation-causing agents, and can give rise to distinctive mutational signatures[7]. It will be interesting to see what can be learnt by applying Aitken and colleagues' methods to analysing chemotherapy-treated cancers. Other examples of exposure to mutation-causing agents, such as acute radiation exposure or sunburn, might also be worth analysing using a strand-by-strand approach. Furthermore, such analysis could clarify the timeline of exposure to a mutation-causing agent: a single, large exposure might generate a mutational signal that could be assigned to individual DNA strands, whereas repeated exposures might cause a progressively less distinct signal. Similarly, analysing individual strands might provide insight into the rate of lesion-repair processes, and offer a new means of studying defects in DNA-repair processes in cancer, such as defective-mismatch repair.

Tracking individual DNA strands is a reductive approach offering a powerful way to study DNA replication and repair processes that have been challenging to observe. Aitken and colleagues' study shows the potential of this method for tackling the complexities of cancer — it seems that the maxim that the whole is greater than the sum of the parts does not apply to individual DNA strands.

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