How would strengthening the connection between brain regions more typically known for their roles in sensory processing lead to a reduction in fear behaviour? Baek *et al.* observed that the combined extinction and ABS procedure dampened the excitability of a population of neurons in the basolateral nucleus of the amygdala (BLA) — an area of the brain that calibrates fear responses[7] — that fired when mice exhibited fear behaviour. They then showed that there is a functional, two-step inhibitory connection between the mediodorsal thalamus and the 'fear-encoding' BLA neurons. When these BLA neurons were optogenetically silenced, the fear-reducing effects of combined extinction and ABS were lost. These findings, put together, suggest a model in which the extinction and ABS procedures act in tandem to recruit the neuronal pathway that links the superior colliculus and the mediodorsal thalamus. This, in turn, reduces the fear response to the trauma-reminding stimulus that is generated by the BLA.

Baek and colleagues' findings paint a comprehensive picture of one of the main neural circuits that underlie the fear-reducing effects of combining extinction and ABS, albeit in a simplified model system. Yet key questions remain. Given that exposure to alternating bilateral visual stimuli is required for memory extinction, it is important to clarify exactly how a mouse, freely moving around the test chamber, perceives these stimuli. Future studies could fix the mouse's head position relative to the LEDs to ensure that the animal's gaze is directed at the alternately flashing lights.

A broader question is how ABS, and by extension EMDR, works to aid memory extinction and reduce fear. One interpretation is that visual stimuli serve as distractors, drawing attention away from the fear-inducing stimulus to dampen anxiety and enable encoding of the extinction memory. But that does not explain the authors' observation that flashing LEDs in a non-sequential pattern fails to reduce fear behaviour. An explanation based on distraction would also sit uneasily with the current view that the process of extinction is enhanced by directing more, not less, attention to the fear-inducing stimulus, because this increased attention reinforces the new connection between the trauma reminder and safety[8].

Baek and colleagues propose that ABS shifts the balance between competing brain circuits, engaging one set of neural pathways that favour fear extinction to overshadow the influence of other pathways that favour the persistence of fear. Whether or not their model turns out to be correct, this study provides a plausible neurobiological explanation for the behavioural effects of ABS — and possibly, by extension, of EMDR. At the very least, this gives us a tractable foundation for further studies of this enigmatic behavioural therapy. Given the pressing need to provide people who have trauma-related illnesses with a range of effective treatment options, this is a most welcome development.

There has been much debate over whether the current technologically driven revolution in neuroscience can help to usher in a new era in treating psychiatric illness. Fulfilling this promise is an enormous challenge, not least because modelling psychiatric disorders and the psychotherapies used to treat them in the laboratory remains difficult[9]. Key features of trauma-related illness, such as learned fear and fear extinction, can be found even in simple organisms, which makes it possible to map their neural bases in detail. Therefore, trauma-related illnesses might represent a great opportunity for therapeutic research[10]. Insights such as those provided by Baek *et al.* offer further encouragement that we will soon see some genuine breakthroughs in how we diagnose, treat and ultimately prevent these devastating conditions. ■

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MEDICAL RESEARCH

Predicting progression of pre-invasive cancer

Early-stage cancerous growths can look similar under the microscope, and whether they will form an invasive tumour is hard to predict. Genomic profiles of these growths in the human lung now enable such a prediction to be made.

HEIDI GREULICH & ANDREW D. CHERNIACK

A type of non-invasive cancer called carcinoma *in situ* (CIS) can occur in the human lung. Some cases of CIS will progress to form an invasive type of cancer known as lung squamous cell carcinoma (LUSC), but until now no method had been developed that could reliably identify which CIS growths would progress. Writing in *Nature Medicine*, Teixeira *et al.*[1] report their analyses of CIS samples from human lung tissue and the identification of a set of genomic alterations that can be used to predict whether CIS is likely to progress to form an invasive tumour.

Biopsy sampling of CIS growths is possible during bronchoscopy surveillance of patients' lungs. Teixeira and colleagues used such biopsies to study the development of LUSC by monitoring CIS growths over time using imaging and by taking cellular samples of a given CIS at different time points. In the people they studied, a subset of the CIS growths either progressed to form LUSC or regressed and regained a normal appearance (Fig. 1). The authors focused on 129 CIS biopsy samples that had been obtained from 85 people before visible signs of progression or regression had been detected. They performed a range of genomic analyses on different subsets of these samples, including whole-genome DNA sequencing, analysis of RNA expression and profiling of a DNA modification called methylation that can influence gene expression.

In the whole-genome DNA-sequencing analysis of 29 samples from individuals whose CIS progressed to LUSC and of 10 regressive CIS samples, the authors found that, overall, the progressive samples had significantly more mutations and more alterations in the number of copies of some genes than the regressive samples had. The most striking finding was that, unlike the regressive samples, almost all of the progressive samples had mutations in the gene *TP53* — a tumour-suppressor gene that helps to prevent the development of cancer. In addition, the progressive samples had a distinct pattern of chromosomal amplifications and deletions of sequences that are commonly found in squamous-cell carcinomas[2] (tumours that originate from cells in tissues that line internal body cavities). The CIS samples that regressed generally lacked notable chromosomal aberrations. Remarkably, of four CIS growths that had *TP53* mutations, many copy-number alterations and that were initially classified as regressive by visual monitoring of the

lungs, three subsequently progressed.

Using a statistical method of data analysis called principal component analysis, Teixeira and colleagues demonstrated that the DNA-methylation patterns in most of the regressive CIS samples were more similar to those of normal lung cells than to the patterns in progressive tumours, except for the cases in which tumours that were initially classified as regressive later progressed. The authors' method could distinguish between regressive and progressive CIS samples using either data for DNA methylation or gene copy number, and the results were consistent regardless of which of these two types of data were used. This indicates that the methylation patterns characteristic of progressive CIS might be influenced by underlying mutations and changes in gene copy number that are associated with progressive CIS.

The authors developed a profile of gene-expression signatures, sets of specific methylated DNA sequences and copy-number alterations for specific genes that could be used to accurately determine the probability of CIS progression. They confirmed the predictive value of their approach by analysing CIS biopsies that had previously been set aside to validate their results. The authors also tested their predictive system by analysing DNA-sequence data from human lung samples obtained from The Cancer Genome Atlas (TCGA) project. They found that their approach could distinguish most LUSC tumours (those likely to be similar to progressive CIS) from the control samples of normal lung tissue (which would be more similar to regressive CIS). Many of the genes for which there were differences in expression and DNA-methylation patterns between progressive and regressive CIS are involved in lung cancer. Teixeira *et al.* also observed differences in expression and methylation of genes associated with chromosomal instability, which is characterized by structural alterations such as large insertions or deletions of DNA.

An important unanswered question concerns how lung CIS growths progress to become LUSC. The authors can identify which CIS growths will progress, but do not know which changes are essential for an invasive cancer to form. The mutation frequencies of key cancer-driver genes in the people who had progressive CIS in Teixeira and colleagues' study are largely similar to the mutation frequencies of these genes in the LUSC tumours in the TCGA database. However, there are some notable differences. For example, a tumour-suppressor gene called *KMT2D* is reported[3] to have a significant association with LUSC, but Teixeira and colleagues found fewer cases of inactivating mutations in this gene associated with progressive CIS than were associated with LUSC tumours in the TCGA database. This discrepancy raises the question of whether *KMT2D* inactivation is a crucial step in the development of LUSC.

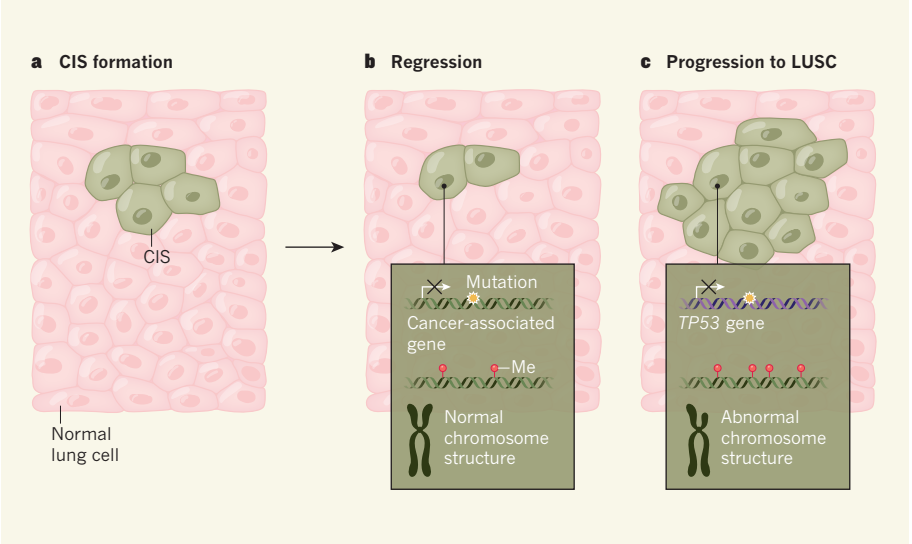

Figure 1 | Hallmarks of invasive lung tumours. **a**, A type of abnormal cellular growth called carcinoma *in situ* (CIS) can occur in the human lung. **b**, **c**, Lung CIS can regress or it can progress to form a malignant invasive cancer known as lung squamous cell carcinoma (LUSC). It is hard to predict which of these paths a CIS will follow, and this has implications for decisions on clinical treatment. Teixeira *et al.*[1] report their analysis of CIS samples taken from patients over time. They report that patterns of gene expression, patterns of a type of DNA modification called methylation (Me) and alterations in chromosomal structure, such as deletions of DNA sequences, could be used to predict whether a CIS would progress to form an invasive cancer. The authors report that a CIS can regress despite the presence of mutations in cancer-associated genes, and that a mutation in the gene *TP53* is a common hallmark of CIS growths that progress.

In addition to mutations of individual genes, another type of DNA alteration that can be a driver of LUSC and of other cancers[3] are small regional (focal) alterations to chromosomes that increase the number of cancer-promoting genes or delete tumour-suppressor genes. Teixeira *et al.* report broad patterns of gene copy-number changes, but don't report on focal alterations, and so whether this type of alteration is involved in the acquisition of invasive characteristics by CIS is unknown. An issue for future study is which molecular mechanisms drive the regression of lung CIS, because some genetic alterations characteristic of CIS were still present in the growths that regressed.

"The authors' method could distinguish between regressive and progressive carcinoma in situ."

The evolution of CIS to form an invasive carcinoma has also been studied in breast cancer. In general, CIS growths in the ductal region of the breast have many of the same types of alteration found in invasive breast tumours[4]. Intriguingly, mutations known to promote the development of a skin cancer called melanoma and of oesophageal cancer have been identified[5–7] in samples of normal skin and oesophagus, respectively. Together with Teixeira and colleagues' results, these findings confirm that the accumulation of all the genomic alterations required for invasive cancer to develop takes time, and they suggest that we might all be carrying the burden of cancer-associated mutations in our seemingly healthy tissues.

Teixeira and colleagues' study sample size was too small for rare genomic alterations to be detected. It would therefore be useful to follow it up with a larger study, which might enable the identification of other genomic alterations involved in the progression of lung CIS to LUSC.

As well as providing the first reported whole-genome-sequencing analysis of lung CIS, Teixeira and colleagues' work offers a glimpse of a future in which precancerous growths that look similar under the microscope could be evaluated on a molecular level to accurately estimate the likelihood that they will develop into invasive cancer. This would enable physicians to tailor therapeutic interventions to the probability of disease progression. ■

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