Taken in isolation, it would be tempting to conclude that this paper dismisses the idea that marine heatwaves are an ecologically important phenomenon. That would be a mistake. Fredston and colleagues' results do not negate the hundreds of papers that have documented ecological consequences of such heatwaves. But how can this negative result be reconciled with almost everything published previously in this realm?

Previous work on the effect of marine heatwaves has focused mainly on the most visible responses. Organisms that suddenly appear thousands of kilometres away from their home range, and mass die-offs of fish and seabirds, are eye-catching and garner much attention from scientists and the public. There might, therefore, be a bias in scientific research towards examining these extreme events that are not representative of more general processes. Fredston and colleagues' analysis circumvents this problem by looking for effects in an ecosystem-level data set that comprises many heatwaves, and the authors therefore gain a different perspective.

The authors' analysis might be criticized for being too broad and generic. For example, the implicit assumption of a common response to marine heatwaves across all ecosystems might not be valid (and is certainly not supported by the evidence available). How individual ecosystems respond to these heatwaves will reflect the unique grouping of the species present and their ability to tolerate extreme temperatures. Ecosystems comprising different species might give different responses to the same heatwave conditions.

The authors also tested for the effect of heatwaves at the individual ecosystem level (thereby relaxing the assumption of a common response), but did not find any statistically significant effects. However, it is worth noting that the amount of data available to make such tests is substantially reduced compared with the analysis across many ecosystems, and the statistical power of the analysis is therefore lower – strong signals might still be there but hidden in the 'noise' of individual ecosystems.

Furthermore, this work focuses solely on fish species that are caught on or close to the bottom of continental shelves (sites located near land and less than 500 metres deep). The effects of heatwaves have been reported for many other groups of organisms, including coral reefs, kelp forests, surface-dwelling (pelagic) fish, marine mammals, seabirds and species that dwell in the sea bed (benthic species)[6]. The sensitivity of each of these groups to marine heatwaves might differ from that of bottom-dwelling fish, reflecting their differing abilities to tolerate (and potentially adapt to) extreme temperatures.

Fredston and colleagues' work reshapes our understanding of how marine systems are affected by heatwaves. Although heatwaves clearly have striking effects in some individual cases, the authors find no evidence for large systematic effects at the community-level for bottom-dwelling fish. Future work needs to address the processes that drive striking effects for some species, but not for others – particularly given that marine heatwaves are becoming more common in a changing climate[8]. As ever in science, the downfall of one hypothesis will give rise to many more questions to answer.

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Medical research

Calligraphy clues to pancreatic cancer origins

Filip Bednar & Marina Pasca di Magliano

Understanding the processes that lead to tumour formation in the pancreas might help in efforts to develop therapies. A new bioinformatics tool called Calligraphy analyses cell–cell signalling to provide fresh insights into how tumours arise.

The process by which normal cells transform into cancer remains unclear. Writing in *Science*, Burdziak *et al.*[1] solve a piece of the puzzle for how such a transformation occurs during the onset of pancreatic cancer.

The pancreas is a complex organ that serves two main functions, each associated with a specific cellular compartment. One – the endocrine pancreas – is formed by structures called the islets of Langerhans, and helps the body to regulate glucose. The other compartment, the exocrine pancreas, includes acinar and ductal cells (which are both a type of epithelial cell), that, respectively, produce digestive enzymes and line the tissue that transports these enzymes to the digestive tract.

The most common form of pancreatic cancer – pancreatic ductal adenocarcinoma – originates from the exocrine pancreas. Pancreatic cancer is a deadly malignancy that bears distinct genetic alterations, most commonly those resulting in cancer-promoting versions of the gene *KRAS* (called oncogenic mutations)[2].

On the basis of this knowledge, genetically engineered mouse models have been designed to express oncogenic *KRAS* in the epithelial cells of the pancreas[3]. Although most of these animal models express oncogenic versions of *KRAS* throughout the pancreas, they only sporadically develop premalignant lesions – known as pancreatic intraepithelial neoplasia (PanIN) – that are composed of altered epithelial cells. The induction of pancreatic inflammation, called pancreatitis, joins forces with oncogenic *KRAS* to drive widespread PanIN[4,5].

Burdziak and colleagues used a combination of biological and computational approaches to understand how early lesions form in the normal pancreas. Furthermore, the authors set the stage to interrogate the progression from early lesions to signs of overt malignancy.

Mouse models of PanIN formation have been described for more than 20 years[6]. However, the advent of technologies for single-cell analysis has enabled researchers to re-examine the process through which normal epithelial cells of the pancreas become malignant. The conventional progression model, colloquially described as PanINgram[7], is based on evaluation of the lesions using histology and genetic-characterization approaches. It also relies on determining whether differentiated acinar or ductal cells of the pancreas revert from their differentiated state to form a duct-like progenitor cell in a process known as acinar-ductal metaplasia (ADM). (Acinar origin is prevalent in mice.) Over time, ADM gives rise to PanIN and, after genetic events such as the loss of tumour-suppressor genes, to cancer[8].

In the absence of oncogenic *KRAS*, ADM occurs after injury (Fig. 1) and is a transient and necessary part of the repair response. Detailed single-cell analysis of inflammatory injury has revealed multiple transient cell

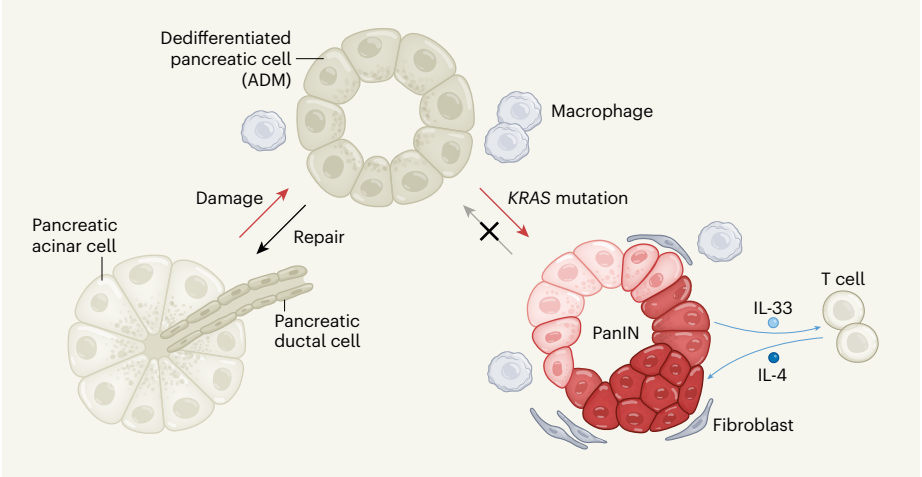

Figure 1 | Insights into the origins of pancreatic cancer. Burdziak *et al.*[1] present a bioinformatics tool called Calligraphy that provides a way to explore cellular interactions as cancer arises. In the normal pancreas, fully differentiated epithelial cells called acinar and ductal cells can revert to 'dedifferentiated' states when damaged; this is a reversible process known as acinar-ductal metaplasia (ADM) and the cells can be repaired. In the presence of cancer-promoting (oncogenic) versions of the *KRAS* gene, either over time or after injury, dedifferentiation instead leads to 'plastic' states (indicated by shades of red) — giving rise to cells called pancreatic intraepithelial neoplasia (PanIN). This is irreversible when oncogenic *KRAS* is activated. This process involves crosstalk between pancreatic epithelial cells and immune cells, such as T cells, through a loop involving signalling molecules called cytokines: IL-33 is derived from pancreatic epithelial cells and IL-4 from T cells. Fibroblast and macrophage cells provide additional signals (not shown).

states, including those with characteristics of tuft, enteroendocrine and duct progenitor cells that secrete mucinous fluid[9]. When oncogenic *KRAS* is activated in the pancreas, only a subset of these injury states are present[9,10].

Burdziak and colleagues took this assessment a step further by combining analyses of gene expression (using single-cell RNA sequencing) and by assessing accessibility of the nuclear DNA–protein complex called chromatin (examined using the technique called single-cell ATAC-seq). This enabled the authors to explore the state of epithelial cells in tissues ranging from the normal pancreas to advanced tumours, and in acute pancreatitis with or without oncogenic *KRAS*.

The authors identify multiple transitional states in epithelial cells; computational modelling also suggests that multiple states can serve as origins for progression to full-blown cancer. This work and previous research from the same group and others[11,12] demonstrate that, mechanistically, oncogenic *KRAS* synergizes with pancreatic-tissue injury to induce altered states of chromatin in epithelial cells, which are then probably co-opted by the transcriptional machinery to complete the transition to cancer.

It is important to note that the current results suggest that only a subset of ADM and PanIN lesions display gene expression and chromatin-accessibility signatures that are associated with advanced-stage tumours. This correlates with observations in people of 'low-grade' versus 'high-grade' PanIN lesions, and implies that only a subset of these lesions have true malignant potential[13]. The synergy of injury-induced inflammation with oncogenic *KRAS* signalling to generate unique chromatin states also supports the idea that each of these stimuli are necessary but not sufficient on their own to cause tumours to form. Indeed, previous work in inducible models of oncogenic *KRAS* expression in mice shows that removing the oncogenic *KRAS* signal from transformed tissues, even in the presence of active inflammation, leads to reversion of cancer formation and tissue recovery[14,15].

It is crucial to underscore the role of the microenvironment in regulating PanIN formation and progression. This is a reciprocal relationship, whereby activation of oncogenic *KRAS* leads to rapid reprogramming of support cells called fibroblasts and infiltration of immune cells into the pancreas. At the same time, inflammation (such as chronic or acute pancreatitis) synergizes with oncogenic *KRAS* to drive PanIN formation.

Burdziak and colleagues investigate this link in greater detail by developing a sophisticated bioinformatics tool to query cell–cell interactions, which they named Calligraphy. Previous computational tools in this realm have focused mainly on individual pairs of receptors and the molecules that they bind, and have been used to propose altered communication relays between the tumour epithelial cell and other cells in the tumour microenvironment. Calligraphy builds on this idea by leveraging the concept of 'communication modules', which are sets of signalling molecules, rather than individual pairs — which are co-expressed — to define modular relays between epithelial cells and immune cells during the early stages of tumour formation.

Using Calligraphy, the authors infer a role for a reciprocal signalling circuit that uses the proteins IL-33 and IL-4 for communication between epithelial cells of the tumour and immune cells (called regulatory T cells and type 2 innate lymphoid cells) in the tumour microenvironment as cancer develops. Burdziak and colleagues validate this finding using a mouse model of inducible depletion of IL-33 specifically in epithelial cells, and demonstrate notable shifting of epithelial states. These data mirror those of previous papers implicating IL-33 as a key signalling relay in pancreatic cancer[11] and pointing to IL-4 as a crucial reciprocal signal derived from T cells of the immune system[16].

A major population not included in this signalling model is fibroblast cells. These co-evolve with epithelial cells of the tumour and serve as a key signalling mediator that drives tumour evolution[17–19]. Fibroblasts are another source of IL-33 in the tumour microenvironment, and, being a cellular source, might be more abundant than the transitional epithelial states expressing IL-33 (ref. 17).

Incorporating fibroblast signalling modules in Calligraphy will further strengthen the authors' proposed interactions, and could enable the identification of previously unknown signalling relays in pancreatic cancer. Furthermore, extending this approach to understand the transition to advanced disease might identify determinants of PanIN progression in people.

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