Pancreatic cancer

Hidden in plain sight

Anirban Maitra

Pancreatic cancer does not respond to certain anticancer treatments that boost immune responses. A mechanism active in tumour cells that contributes to this evasion of immune targeting has been uncovered. **See p.100**

A consistent hallmark of pancreatic cancer is the inability to treat it with immunotherapy – an approach that harnesses the body's immune response to target a cancer. On page 100, Yamamoto *et al.*[1] reveal a mechanism that enables pancreatic cancer cells to evade an immune response. The process implicated is usually associated with the normal degradation and recycling of cellular proteins. The authors find that inhibition of this pathway, using drugs or by genetic approaches, reverses this immune evasion in animal models of pancreatic cancer. The finding provides a compelling rationale for investigating whether targeting this pathway might be of benefit in the clinic.

The past decade has seen striking advances in the use of immunotherapy to treat numerous solid cancers (those not formed from blood cells) that had not responded to earlier therapy attempts. One such advance is the development of what is known as checkpoint blockade therapy. This targets the proteins PD-1 and CTLA-4, which are found on the surface of immune cells called cytotoxic T cells (also called CD8 T cells) and inhibit an immune response. The administration of two antibodies that, respectively, target these two inhibitory proteins is used widely to drive an antitumour immune response[2]. Unfortunately, treatment of pancreatic cancer with either antibody, or the two together, has not led to any notable success in terms of patient survival[3].

There are many factors that can contribute to the failure of immunotherapy. The tumour microenvironment of pancreatic cancer contains a variety of immune cells that suppress the function of cytotoxic T cells – they include myeloid cells, tumour-associated macrophages and regulatory B cells[4]. Moreover, signals released by the cancer cells themselves (often mediated by the aberrant activation of tumour-promoting genes such as those encoding the proteins Ras and Myc) have a key role in creating this profoundly immunosuppressive environment[5]. In addition, fibroblast cells in the tumour microenvironment secrete material that generates a physical barrier hindering the influx of T cells to the tumour site[6].

Another equally crucial cause of immune evasion is that the cancer cells themselves undergo changes. An immune response is triggered when a T cell recognizes as foreign a peptide fragment (called an antigen) that is 'presented' on the surface of a cancer cell, bound to a molecule called a major histocompatibility complex (MHC) class I molecule (Fig. 1). An inability of the MHC molecules to present tumour antigens has emerged[7] as an explanation for how cancer cells can hide from the immune system. These molecules comprise an invariant β_2-microglobulin protein (encoded by the *B2M* gene) and a protein encoded by HLA genes, which vary in the antigen-binding site they encode, thereby enabling different antigens to be bound by different MHC molecules. Tumour-derived-antigen presentation by MHC class I molecules is required for cancer cells to be recognized by the T-cell receptor on cytotoxic T cells.

The inability to present antigens, and thus the absence of immune recognition, can occur through alterations in the genes encoding proteins needed for antigen presentation, such as *B2M* and HLA genes[8,9]. In pancreatic cancer, such genetic alterations are relatively uncommon, occurring in no more than 1% of cases[10]. However, lower-than-normal levels of MHC class I molecules, or their complete loss, occurs in more than 60% of cancers that arise in the pancreas[11], and the decrease might be even greater in metastatic tumours (those that have spread beyond the pancreas)[7]. Until now, the mechanisms underlying the regulation of MHC class I molecules in pancreatic cancer have remained elusive.

Yamamoto and colleagues reveal that a cellular pathway called autophagy is the means by which pancreatic cancer cells limit the amount of MHC class I molecules on their surface, thereby hindering antigen

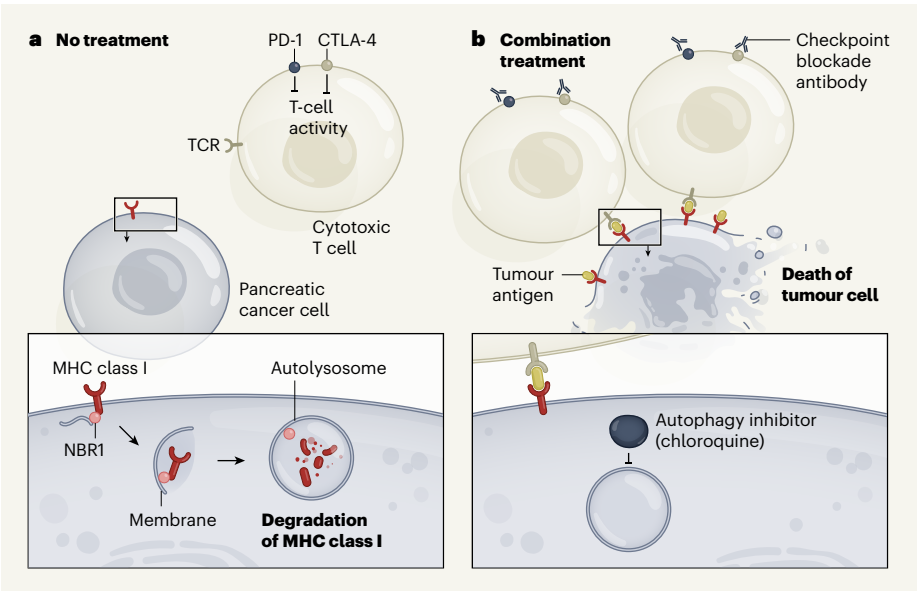

Figure 1 | Boosting the targeting of pancreatic cancer by the immune system. The ability of immune cells called cytotoxic T cells to attack tumours is thwarted by, for example, the action of inhibitory proteins called CTLA-4 and PD-1. Yamamoto *et al.*[1] report a previously unsuspected mechanism that enables pancreatic cancer to evade immune cells. **a**, The T-cell receptor (TCR) on the surface of cytotoxic T cells enables them to recognize tumour cells, and this recognition depends on the presence of major histocompatibility complex (MHC) class I molecules on the surface of cancer cells. Yamamoto and colleagues report that MHC class I molecules in pancreatic cancer cells are destroyed by a process termed autophagy. This begins when the molecule binds to the protein NBR1 and is enveloped in a membrane to form an organelle called an autolysosome. The destruction of MHC class I molecules thus prevents them from 'presenting' peptide fragments (antigens) from the tumour that might be recognized by the TCR. **b**, In mouse models of pancreatic cancer, treatment combining an autophagy-inhibiting drug (chloroquine) and antibodies targeting PD-1 and CTLA-4 (termed checkpoint blockade therapy) provoked a robust immune response against the tumour compared, with the case for animals that did not receive such treatment.

presentation. Autophagy is an essential cellular degradation pathway that recycles organelles and proteins to maintain cellular 'fitness'[12]. It can act selectively through the binding of a specific receptor to a 'cargo' (for example, a protein or organelle) that has been targeted for destruction by being marked with a tag, such as the protein ubiquitin. A complex of receptor and cargo is enveloped in a lipid membrane to form a vesicle called an autophagosome, which fuses with an organelle known as a lysosome to form an autolysosome (Fig. 1). The cargo then undergoes enzyme-mediated digestion in the autolysosome and its contents are recycled for use in the cell.

Yamamoto *et al*. report that, remarkably, in pancreatic cancer cells, most MHC class I molecules do not exist on the cell surface, but instead are found in autophagosomes and autolysosomes. The authors identified an autophagy-associated receptor called NBR1 as being responsible for targeting MHC class I molecules to the autophagy machinery. Furthermore, they found that if autophagy was inhibited in mice, by drugs such as chloroquine or through genetic engineering, this restored the surface expression of MHC class I molecules, thereby enhancing antigen presentation. In mouse models of pancreatic cancer, autophagy inhibition resulted in an influx of cytotoxic T cells to the tumour microenvironment, and if the animals also received checkpoint blockade therapy, a robust antitumour immune response was generated.

Increased autophagy has been known for around a decade[13] to be a metabolic requirement for pancreatic cancer, but only now has a connection been made to the immune evasion of tumour cells. Thus far, clinical trials targeting autophagy in pancreatic cancer have relied on testing the antimalarial drug hydroxychloroquine (chloroquine and hydroxychloroquine are related molecules), which blocks one of the final steps in autophagy. Other drug candidates, directed at earlier components of the autophagy machinery, are in the pipeline. Early trials of hydroxychloroquine demonstrated only modest results, but there has been a resurgence of interest in combinatorial treatment approaches after evidence from animal models that, if signalling mediated downstream of mutant Ras by the enzyme MAP kinase is inhibited, pancreatic cancer cells become strongly dependent on autophagy for their survival[14,15].

Yamamoto and colleagues' work will almost certainly lead to further additions to the compendium of autophagy-targeted clinical trials of pancreatic cancer treatments. Discoveries in the fields of autophagy and immunotherapy were, respectively, recognized by the Nobel Prize in Physiology or Medicine in 2016 and 2018. This new finding represents an unprecedented opportunity for the convergence of these two areas of study, in efforts to improve therapies for pancreatic cancer.

Anirban Maitra is in the Sheikh Ahmed Center for Pancreatic Cancer Research, University of Texas MD Anderson Cancer Center, Houston, Texas 77005, USA.
e-mail: amaitra@mdanderson.org

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Precision measurements

Mass spectrometry for future atomic clocks

Marianna S. Safronova

Highly charged ions could form the basis of the next generation of ultra-precise clocks, using electronic transitions in the ions as the 'pendulum'. An ingenious method for characterizing such transitions has been reported. **See p.42**

Atomic clocks, which use transitions between the energy levels of electrons in atoms as a reference for their timekeeping mechanism, are the world's most accurate clocks — they will not lose one second during the lifetime of the Universe[1]. This means that they can be used in ultra-precise measurements to probe some of the fundamental postulates of modern physics. Clocks based on highly charged ions (HCIs; atoms from which many electrons have been removed) are predicted to have even more sensitivity in these investigations[2]. However, the development of such clocks is hampered by the difficulty of detecting suitable transitions in HCIs.

"The authors used Einstein's principle of energy–mass equivalence to convert a mass measurement into an energy measurement."

On page 42, Schüssler *et al.*[3] report that they have measured a long-lived, excited electronic state in a highly charged rhenium ion using the mass difference of the ion in its ground and excited states. This non-destructive, direct determination of an electronic excitation in an HCI will aid the discovery of HCI transitions that would be suitable for use in a clock.

To build a clock, one needs a periodic event whose frequency acts as a reference for timekeeping. Electronic transitions in atoms are perfect natural oscillators for this purpose. An ultra-stable laser must be tuned to the exact frequency of the atomic transition to drive the oscillation, much as a musical instrument must be tuned to produce the right tone.

Can just any atomic transition be used? No — suitable transitions are hard to come by. The best transitions start from the lowest energy state of an atom (the ground state) and must end up in a long-lived (metastable) excited state. The energy needed to stimulate the transition must also be within the range of tabletop-laser technologies.

Moreover, the atoms must be held in traps, so that their motion is almost completely frozen — in other words, the operation of atomic clocks requires precision manipulation of quantum systems. For this reason, currently available clocks use transitions either in electrically neutral atoms or in ions produced by removing one electron from an atom, because these systems are the most amenable to precision quantum control.

Substantial advances have been made in studies of HCIs, and all the technologies required to make a clock using such an ion were demonstrated only this year[4]. However, progress is hindered by the difficulty in using conventional atomic spectroscopy to identify and measure transitions suitable for use in clocks — the characteristics of such transitions mean that they are, by definition, very weak (the probability of the transition occurring