Tumour biology

Immune cells alter genetic decoding in cancer

Pavel V. Baranov & John F. Atkins

Cancer cells make proteins in which the amino acid phenylalanine is swapped for tryptophan when immune cells trigger a tryptophan shortage. This finding reveals unexpected dynamics of genetic decoding. **See p.721**

Understanding the characteristics that are unique to tumour cells offers a potential way to develop new anticancer therapeutics. On page 721, Pataskar *et al.*[1] report an unusual twist in the way that certain cancer cells make proteins.

There is growing interest in pinpointing distinctive features and interactions between tumour cells and other components of their microenvironment, such as immune cells. For example, in the vicinity of a tumour, the activated form of an immune cell called a T cell combats cancer by secreting the signalling protein interferon-γ, which is perhaps best known for its role in antiviral defence.

Cancer cells respond to interferon-γ by driving expression of an enzyme that uses the amino acid tryptophan. The supply of tryptophan in cancer cells is therefore greatly diminished compared with that in non-cancerous cells[2]. Using human cancer cells grown *in vitro*, Pataskar and colleagues investigated the consequence of this interferon-γ-mediated tryptophan shortage. They engineered the cells to express versions of DNA encoding green fluorescent protein (GFP). This DNA contained a three-nucleotide sequence called a codon that normally results in the incorporation of tryptophan into the protein. Unexpectedly, the authors found that the cells still synthesized the protein despite tryptophan depletion.

Pataskar and colleagues discovered that the persistence of protein production in the absence of tryptophan was due to a surprising switch. Under conditions of tryptophan sufficiency, the cellular component — a transfer RNA — that recognizes tryptophan-encoding codons is uniquely 'linked' to tryptophan, and thus provides this residue for incorporation into the growing chain of amino acids during the translation of messenger RNA. However, the authors' evidence indicates that, under conditions of tryptophan depletion, the amino acid phenylalanine, which is structurally similar to tryptophan, instead becomes 'linked' to the tRNA that recognizes codons for tryptophan. As a result, during the decoding of a tryptophan codon, phenylalanine is specified instead of tryptophan, altering the usual 'meaning' of the codon (Fig. 1). The authors provide evidence that this striking reassignment of codon meaning occurs in multiple types of cancer cell.

The misincorporation of phenylalanine is a double-edged sword for cancer cells. On the one hand, it allows the cells to evade protein-synthesis problems owing to tryptophan shortage. They continue to synthesize full-length proteins despite missing an essential amino acid, although the forms of these proteins are different from usual as a result of the amino-acid substitutions. Because phenylalanine is similar to tryptophan, the alterations in the protein 3D structures are presumably less severe than those that would have occurred with substitutions of most other amino acids. Thus, the cells survive.

On the other hand, the body has not encountered these modified versions of proteins before, and so they would be expected to be recognized as foreign by immune-system defences. Pataskar *et al.* report that protein fragments termed antigens that contain these tryptophan-to-phenylalanine 'substitutants' (as the authors call them) are indeed displayed on the surface of the cells for presentation to immune cells, raising the possibility of triggering an immune response. Therefore, the same substitutants that help cancer cells to overcome the impairment in their protein-production ability provide a beacon that flags these cells as a threat, necessitating their elimination by the immune system. These findings reveal a previously unknown link between amino-acid availability and the immune response that should be explored for its potential use in cancer immunotherapy, and maybe even for anticancer dietary interventions.

Despite the structural similarity between phenylalanine and tryptophan, such substitutions would nevertheless be expected to alter the structure of numerous proteins and impair their function and turnover. Examples are known of cases in which such substitutions reduce enzymatic activity[3]. It is also theoretically possible that substitutants can enable certain proteins to function in new ways, for example by changing enzyme

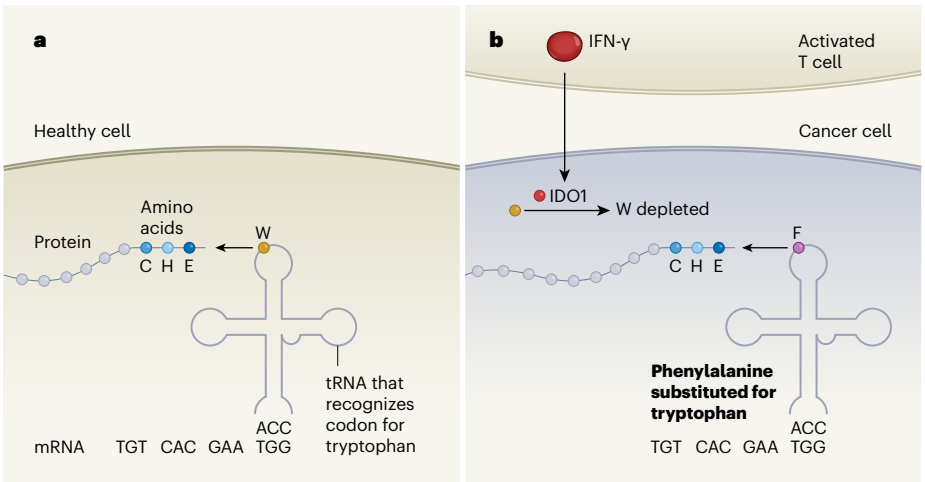

Figure 1 | Cancer cells break the normal rules of protein synthesis. a, In healthy cells, normal deciphering of the genetic code means that a particular nucleotide triplet (termed a codon) of messenger RNA (mRNA) results in the reliable incorporation of a specific amino acid — such as the amino acids termed C, H and E in the single-letter code for amino acids — into a protein being made. For example, the codon TGG is recognized by a transfer RNA (tRNA) with a matching complementary RNA sequence (ACC), and this tRNA provides the amino acid tryptophan (W in the single-letter code). **b**, Cancer cells can encounter tryptophan shortages mediated by a type of immune cell called an activated T cell. This occurs when the protein interferon-γ (IFN-γ) drives expression of the enzyme IDO1, which uses up tryptophan. Pataskar *et al.*[1] report that cancer cells can overcome this challenge by swapping the amino acid incorporated when a codon for tryptophan occurs. The tRNA that recognizes the codon for tryptophan becomes joined instead to the amino acid phenylalanine (F in the single-letter code). This alters a protein's amino-acid sequence, and might thus affect the protein's structure and function.

substrate-recognition domains or enabling binding to different proteins from the usual ones. It will be important to explore how the properties of individual proteins are altered by these substitutions and how it affects cancer cells.

This substitution phenomenon has not been found in non-cancerous cells, nor in some types of cancer cell. The authors suggest that differences in the immune-cell microenvironments surrounding tumours are responsible for this difference between cancer cells. However, the lack of identification of the key factors underlying the generation of the substitutants is a limitation of this study. Pinpointing the molecular components involved and the specific conditions required for substitutants to arise is a crucial task for the future.

Pataskar and colleagues' discovery adds to a growing appreciation of the diversity and dynamic nature of genetic decoding[4]. The genetic code (the 'rule book' that defines the relationship between codons and amino acids) is not universal; many variants are known, including those used by our own mitochondrial organelles, and new variants continue to be discovered[5–7]. The change of a codon assignment in the genetic code is thought to be a slow evolutionary process involving either the loss of a specific codon from the entire genome or its ambiguous decoding as either one of two possible amino acids over a long period of time. Once changed, such an alteration is generally thought to be hard-wired, and most proteins in a cell are synthesized according to the genetic code of that cell, with a small number of exceptions at specific regions of protein-coding sequences (such as during the incorporation of non-standard amino acids, or as a result of a process called ribosomal frameshifting)[8].

However, instances of low-efficiency incorporation of the 'wrong' amino acids into proteins have been observed in many microorganisms. This deviation is thought to allow the microbes to diversify their complement of proteins and thereby adapt to stressful conditions, and it occurs particularly often during infection of a host. For example, species of the disease-causing bacterium *Streptomyces* use partial switching of a specified amino acid to aid their invasion of plants[9]. From the host's side, in response to viral attack, mammalian cells increase the random misincorporation of a sulfur-containing amino acid into their proteins[10]. Such an amino acid is more likely to react with and neutralize reactive oxygen species. This process is thought to buffer proteins against the damage mediated by reactive oxygen species that are generated by the infection. However, the example that Pataskar *et al*. discovered is striking because it is a more efficient and more specific response to a cellular challenge.

Are other examples of conditional changes to the genetic code's rule book awaiting discovery? We might know the answer reasonably soon. Freely available data sets providing results generated by an approach called mass spectrometry could be explored to determine whether such proteomics information indicates that a rise in specific amino-acid substitutions is associated with a particular condition or disease. Given the large number of data sets already available, it is surprising that substitutants were not discovered earlier. One possible reason, as noted by the physicist Richard Feynman, is that the human imagination is limited compared with that of nature. However, once one example is discovered, it does not require a lot of imagination to search for others.

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The authors declare no competing interests.
This article was published online on 9 March 2022.

Chemical biology

Reversible protein inhibitors kept on target

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Compounds that form reversible covalent bonds with lysine amino-acid residues in proteins have high potential for drug discovery. A chemical group has been reported that prolongs the time for which such compounds bind to their targets.

In the past few years, drug-discovery researchers have become increasingly interested in covalent inhibitors — compounds that bind and inhibit a target protein by forming a covalent bond to it[1,2], rather than binding through non-covalent interactions alone, as most conventional small-molecule drugs do. A subclass of compounds known as reversible covalent inhibitors has particular promise, but the chemical groups available for their design can pose the challenge that they do not bind to proteins for long enough to exert an effect. Writing in the *Journal of the American Chemical Society*, Reja *et al.*[3] report a chemically reactive group that targets commonly found lysine amino-acid residues in proteins, and that prolongs the time spent by reversible covalent inhibitors at their binding sites.

Covalent inhibitors have attracted interest for three main reasons. First, they can have a higher binding affinity for proteins than do conventional drugs, which allows them to target shallow binding sites. Second, they have potentially longer durations of action than conventional drugs, which can lead to less-frequent drug dosing. And third, they can be targeted specifically to just one out of a group of closely related proteins[1].

Inhibitors that form irreversible covalent bonds to their targets have had many successes in drug development, with some now approved for use in the clinic[2]. However, the reactivity and selectivity of irreversible covalent inhibitors need careful tailoring to prevent these compounds from becoming permanently attached to off-target proteins, because such attachment can cause toxicity or allergies[1]. Compounds that form reversible covalent bonds offer a solution to this problem, because they can potentially disengage from off-target proteins. Nevertheless, precise fine-tuning of the kinetics of binding to, and dissociation from, proteins is still needed to ensure that reversible covalent inhibitors reside at the target protein for long enough to exert an effect.

Groundbreaking work on reversible covalent inhibitors has produced compounds that target the thiol (SH) group of non-catalytic cysteine residues in kinase enzymes[4]. However, cysteine is relatively rarely found in the proteome[5] (the complete set of proteins encoded by a genome), so it would be useful to develop reversible covalent inhibitors that form bonds to other amino-acid residues, to broaden the applicability of these compounds.

The lysine residue is particularly interesting

Clarification

In Figure 1 of this News & Views, T is being used to represent the RNA building block uridine, as is conventional in some fields.