

## HEART DISEASE

## Death-defying plaque cells

Dead cells are usually removed through their ingestion and destruction by other cells. A study of plaque deposits in arteries shows that dying cells in plaques display a 'don't-eat-me' signal that blocks their removal. [SEE LETTER P.86](#)

IRA TABAS

Heart attacks and strokes, which are leading causes of death worldwide<sup>1</sup>, begin with a process called atherosclerosis, in which plaques — accumulations of lipids, cells, extracellular matrix and cellular debris — occur in certain areas of arteries. Although most people's arteries contain many such plaques, only a small percentage will cause disease<sup>2</sup>. On page 86, Kojima *et al.*<sup>3</sup> provide a plausible mechanism that could explain why some plaques become clinically dangerous.

A key feature of clinically dangerous ('vulnerable') plaques is a structure called the necrotic core, which contains dead cells that have undergone a type of cell death known as necrosis. The necrotic core is inflamed and has a thinning fibrous cap that covers the plaque and separates it from the central lumen of the artery<sup>2</sup> (Fig. 1). When the cap ruptures or erodes, the necrotic material becomes exposed to circulating blood-cell fragments called platelets that are necessary for blood clotting. This exposure results in platelet aggregation

(thrombus), which may block the blood vessel and thereby cause a heart attack or stroke by depriving the heart or brain of oxygen. The necrotic core, which harbours inflammatory cellular debris, promotes cap disruption by contributing to the degradation of the cap's structural protein, collagen, and by creating physical stress on the cap<sup>4</sup>. Understanding how the necrotic core develops is an urgent goal in heart-disease research.

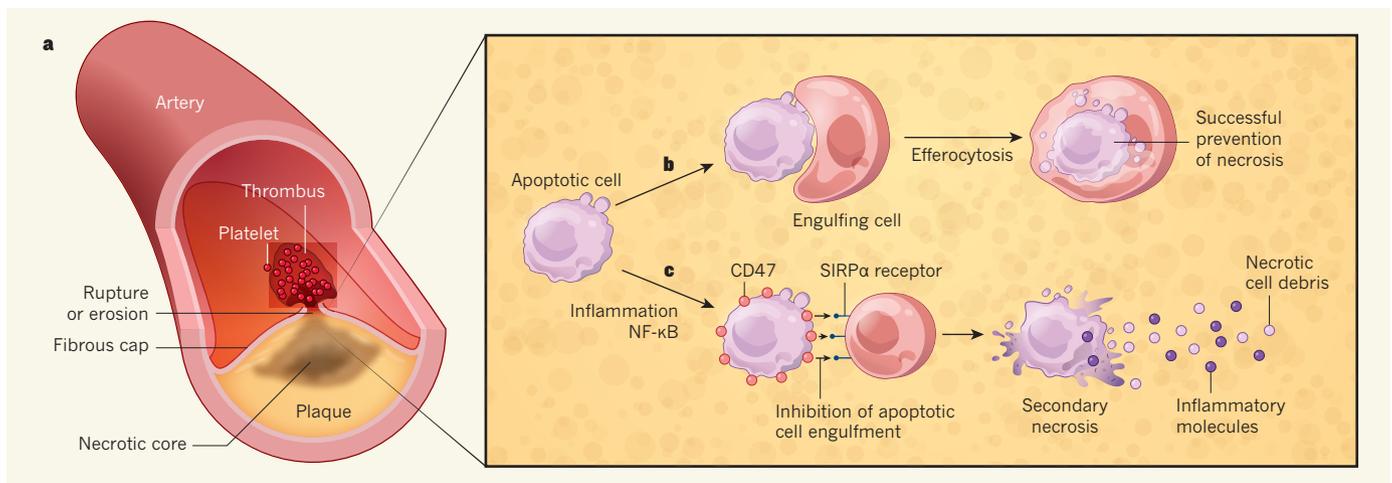
To determine how dying cells in plaques undergo necrosis, it is necessary to understand how the body normally prevents necrotic cell death. Billions of cells in the body die every day through a process called apoptosis, which initially prevents cell-membrane rupture and leakage of inflammatory cellular contents. Apoptotic cells are rapidly and safely removed by an evolutionarily conserved process called efferocytosis, in which the apoptotic cell is internalized and destroyed by an engulfing cell, called a phagocyte, before membrane rupture occurs.

Efferocytosis requires signalling between the dying cell and the phagocyte: factors

produced by the apoptotic cell promote the migration of phagocytes towards apoptotic cells, and 'eat-me' recognition markers on the surface of apoptotic cells are recognized by receptors on phagocytes<sup>5</sup>. As a fail-safe mechanism, healthy living cells often express 'don't-eat-me' molecules on their cell surface that signal to block phagocytes from internalizing a live cell. The CD47 protein is an example of a don't-eat-me molecule that signals through the SIRPα receptor protein on phagocytes to inhibit apoptotic-cell engulfment<sup>5</sup>.

What goes wrong in vulnerable plaques? Studies have shown that efferocytosis is defective in 'advanced' human plaques that have not yet reached the vulnerable stage<sup>6</sup>, and experiments using genetically engineered mice<sup>4</sup> have demonstrated a causal relationship between defective efferocytosis and plaque necrosis. Thus, in advanced plaques, uncleared apoptotic cells eventually become leaky, resulting in a process called secondary necrosis.

Why does efferocytosis become defective in advanced atherosclerosis? Kojima and colleagues provide a plausible mechanism. They



**Figure 1 | Defective removal of dead cells can contribute to clinically dangerous atherosclerotic plaques.** **a**, Many clinically dangerous plaques contain a structure called the necrotic core, characterized by inflammation and necrotic cell death. In atherosclerosis, if the fibrous cap covering the plaque ruptures or erodes, release of material from the necrotic core can trigger platelet aggregation (known as a thrombus) and arterial blockage, which may result in heart attack or stroke. Understanding how plaques develop to a necrotic state is a key question. **b**, Plaque cells undergo a non-inflammatory type of cell death called apoptosis. In asymptomatic

non-necrotic plaques, rapid removal of apoptotic cells by engulfing cells — a process known as efferocytosis — prevents necrosis. **c**, Kojima *et al.*<sup>3</sup> found that the inflammatory conditions of advanced atherosclerosis lead to persistent expression of the protein marker CD47 on plaque cells through the inflammatory-signalling mediator NF-κB. When these cells become apoptotic, CD47 sends a signal through the SIRPα receptor on the engulfing cell to block engulfment. The unengulfed cells undergo a type of cell death called secondary necrosis, leading to the release of inflammatory molecules and the formation of necrotic cores from the cell debris.

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1. World Health Organization. <http://www.who.int/mediacentre/factsheets/fs310/en> (2014).
2. Virmani, R., Burke, A. P., Kolodgie, F. D. & Farb, A. J. *Interv. Cardiol.* **15**, 439–446 (2002).
3. Kojima, Y. *et al. Nature* **536**, 86–90 (2016).
4. Thorp, E., Subramanian, M. & Tabas, I. *Eur. J. Immunol.* **41**, 2515–2518 (2011).
5. Arandjelovic, S. & Ravichandran, K. S. *Nature Immunol.* **16**, 907–917 (2015).
6. Schrijvers, D. M., De Meyer, G. R., Kockx, M. M., Herman, A. G. & Martinet, W. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1256–1261 (2005).
7. Lin, J. *et al. Cell Rep.* **3**, 200–210 (2013).
8. Greenlee-Wacker, M. C. *et al. J. Immunol.* **192**, 4709–4717 (2014).
9. Kojima, Y. *et al. J. Clin. Invest.* **124**, 1083–1097 (2014).
10. Thorp, E. *et al. J. Biol. Chem.* **286**, 33335–33344 (2011).
11. Garbin, U. *et al. Cardiovasc. Res.* **97**, 125–133 (2013).
12. Tabas, I. & Glass, C. K. *Science* **339**, 166–172 (2013).
13. <https://clinicaltrials.gov/ct2/show/NCT02678338>
14. Liu, J. *et al. PLoS One* **10**, e0137345 (2015).
15. Serhan, C. N. *Nature* **510**, 92–101 (2014).
16. Fredman, G. *et al. Sci. Transl. Med.* **7**, 275ra20 (2015).
17. <http://www.businesswire.com/news/home/20090824005320/en/Resolvyn-Announces-Positive-Data-Phase-2-Clinical>

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made the surprising finding that in histological sections from human and mouse plaques, unengulfed dying macrophage and vascular smooth muscle cells display the don't-eat-me signal CD47 on their surface. In a mouse model of atherosclerosis, the authors found that infusion of an antibody that blocks CD47 improved efferocytosis in the plaque and lessened formation of the necrotic core. On the basis of an *in vitro* model, they suggest that CD47 is transcriptionally induced by NF- $\kappa$ B, which orchestrates inflammatory programs in cells, including plaque cells. Defective phagocytic clearance of cells that die by another mechanism — an enzyme-triggered necrotic process called primary necrosis — may also contribute to the formation of the necrotic core<sup>7</sup>, and here too the problem could involve abnormal expression of CD47 (ref. 8).

The complex nature of both atherosclerosis and efferocytosis suggests that multiple mechanisms cause defective efferocytosis as plaques progress<sup>4</sup>. Workers from Kojima and colleagues<sup>3</sup> laboratory previously showed<sup>9</sup> that dead cells in the plaque show a deficit in expression of the eat-me signal calreticulin protein. Moreover, the MerTK receptor protein present on phagocytic macrophages, which mediates efferocytosis in advanced plaques, undergoes degradation in the same type of inflammatory condition in atherosclerosis that Kojima and colleagues suggest leads to expression of CD47. The protease enzyme ADAM17 activates tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), which induces CD47 in vascular smooth muscle cells, and ADAM17 also destroys MerTK<sup>10</sup>. Both ADAM17 activation and cleavage of MerTK have been implicated in the progression of human plaques towards a clinically dangerous state<sup>11</sup>.

How might our knowledge of defective efferocytosis in general, and the insights gained from the work of Kojima and colleagues in particular, lead to future therapies to block the formation of dangerous plaques? Treatment with anti-TNF- $\alpha$  antibodies would block CD47 induction, and this strategy has been successful in debilitating autoimmune diseases for which TNF- $\alpha$  is a dominant trigger, notably rheumatoid arthritis. However, in atherosclerosis, it is probable that inflammation occurs through multiple pathways. Another concern is that anti-TNF- $\alpha$  treatment can compromise immune defences, which would challenge its long-term use as a preventive therapy in mostly asymptomatic people at risk of acute heart disease<sup>12</sup>.

Treatment with anti-CD47 antibodies, which is being tested as a cancer treatment in early clinical trials<sup>13</sup>, presents other challenges. CD47 is used by red blood cells to prevent their premature engulfment before cell senescence, and a major adverse effect of anti-CD47 therapy is anaemia<sup>14</sup> (a decrease in the number of red blood cells). Moreover, CD47 has roles in cell adhesion and migration, so its inhibition

might cause adverse effects related to these functions in processes such as blood-vessel formation and immune defence.

Another therapeutic strategy is based on the observation that many processes that generate vulnerable plaques, including inefficient efferocytosis, can be caused by defects in a biological program known as resolution of inflammation, which normally terminates an inflammatory response when it is no longer needed, and initiates tissue repair. Administration of compounds that mediate this resolution program has proved beneficial in many preclinical models of resolution-defective diseases<sup>15</sup>. For example, such treatment can improve efferocytosis and suppress plaque necrosis in advanced atherosclerosis<sup>16</sup>. Moreover, resolution-mediator therapy may actually boost host defence<sup>15</sup>, and this approach is now being tested in early clinical trials targeting chronic inflammatory conditions<sup>17</sup>. These and other future developments based on work such as that of Kojima and colleagues may some day provide a safe way to keep the plaques in our arteries from becoming clinically dangerous. ■

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#### SYNTHETIC BIOLOGY

# Bacteria synchronized for drug delivery

**A synthetic genetic circuit that mimics the quorum-sensing systems used by bacterial populations to coordinate gene expression enables bacteria to deliver drugs to mouse tumours in repeated and synchronized cycles. SEE LETTER P.81**

SHIBIN ZHOU

**H**umans and bacteria have a long history of parasitic and symbiotic relationships. Now, Din *et al.*<sup>1</sup> exploit a relationship between bacteria and diseased human tissue for a therapeutic purpose. On page 81, the authors outline a system in which engineered bacteria acting as drug-delivery vehicles simultaneously break down, releasing an antitumour drug in synchronized cycles to maximize delivery efficiency and minimize toxicity.

In the body, some niches for bacteria — such as the anaerobic lumen of the intestines — have low oxygen levels. Similar conditions are found in solid tumours because of increased oxygen demand owing to highly proliferative tumour cells and insufficient blood supply owing to a structurally and functionally abnormal tumour vasculature<sup>2</sup>. The hypoxic areas in a tumour are relatively protected from attacks by the body's

immune system, further facilitating bacterial colonization and growth<sup>3</sup>.

The idea of using bacteria to fight cancer has been around for more than a century. In 1891, surgeon William B. Coley infected patients with *Streptococcus* bacteria in an attempt to activate the immune system to fight cancer<sup>4</sup>. The method was controversial because of inconsistent efficacy and the toxicity of streptococcal infection. But the idea resurfaced later, when more was known about the tumour microenvironment and genetic-engineering tools had emerged, raising the hope that more-potent and less-toxic (attenuated) bacterial strains could be generated. Several bacterial strains have now been developed as agents for cancer therapy and they are showing promising effects in experimental models<sup>5</sup>.

Bacteria can destroy diseased tissue by competing for nutrients, secreting toxins and eliciting host immune responses. They