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Finding the culprit in your heart

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Cardiovascular disease, the leading cause of death worldwide, is caused by atherothrombotic vascular occlusion, which leads to unstable angina (progressive ischemia of heart muscle), heart attacks, sudden cardiac death and stroke¹. Atherothrombosis is a focal pathological process in medium-sized arteries, notably those that feed the heart and brain, and comprises two distinct stages. The first involves a decades-long accumulation of lipids, macrophages and other inflammatory cells, and extracellular matrix in the sub endothelial space, or intima, of the blood vessel wall. This process alone does not cause acute cardiovascular events, because blood flow is preserved through outward remodeling of the arterial wall or, in the setting of gradual luminal encroachment, new vessel formation².

However, in a small percentage of lesions, acute, occlusive luminal thrombosis is triggered, leading to ischemia or death of distal organ tissue. When these disease-causing lesions are identified by imaging techniques or at autopsy, they are termed ‘culprit lesions’ because they are deemed responsible for causing the arterial occlusion. But what is different about the lesions that progress to the point that they cause these acute events and end-organ damage compared to those that do not? This question was addressed in a unique clinical study by Stone *et al.*³, which reports findings that have important implications for basic research into culprit lesion formation.

Autopsy studies of subjects that die of an acute atherothrombotic vascular event, such as a massive heart attack, have found that culprit lesions are distinguished not by lesion size but rather by several distinct morphological features, including collections of dead cells, often referred to as ‘necrotic cores’, and erosion or rupture of the scar tissue, or ‘fibrous cap’, that overlies the lesion⁴. But these studies are retrospective and sample only one point in time and thus cannot be used to draw firm conclusions on the process of culprit lesion formation. Prospective analysis would require serial ‘sampling’ of lesions during a period in which new atherothrombotic vascular events occur.

Although serial tissue sampling in humans is not possible, serial imaging of coronary arteries is feasible. In this context, Stone *et al.*³ studied individuals who suffered an acute cardiac event and thus required a coronary artery catheterization procedure to reestablish lumen patency at the site of the culprit lesion. During this therapeutic procedure, the investigators imaged many nonculprit lesions in the coronary artery circulation by combining serial angiography with a technique called intravascular ultrasound (IVUS). After a median follow-up of ~3 years, an acute atherothrombotic clinical event occurred in 135 individuals, who then underwent repeat angiography that identified the sites of the new

thrombosis. Through comparisons with the earlier images, the authors could identify the earlier lesions that gave rise to these new culprit lesions.

As expected, some of the new events were caused by recurrence at the sites of the original culprit lesions. But many events occurred at sites that several years previously were non-culprit lesions. The authors asked whether any features of these ‘preculprit’ lesions predicted clinical progression. Consistent with previous data, most lesions that progressed did not show marked luminal narrowing at the earlier time point, but two features, large necrotic cores and thin fibrous caps—which together characterize ‘thin-cap fibroatheroma’—were highly predictive of progression to culprit lesions and the only independent risk factor for major cardiovascular events (**Fig. 1**).

How do these findings open new avenues in basic research in this area and help us identify new molecular targets for potential therapeutic intervention? Atherosclerosis consists of numerous, heterogeneous cell biological processes, and different lesions in the same individual vary in terms of which processes are dominant. For example, most lesions have many cholesterol-loaded macrophage foam cells, others have fibrous tissue as the major feature and still others have the dangerous features identified in the above study. Initial lesion formation and progression of mostly early- to mid-stage lesions can be prevented, or even regressed, by lowering the concentration of plasma atherogenic lipoproteins⁵. But can more specific therapy be directed at potential time bombs such as the lesions with the highest potential to progress to the culprit stage in high-risk patients? Knowing which lesions among the heterogeneous mix are most likely to progress and the mechanisms involved is crucial, considering that, on average, less than 5% of lesions in individuals at risk progress to the culprit stage⁴.

One key attribute of the lesions identified in the study, intimal necrosis, forms as a consequence of primary or secondary necrotic death of macrophages⁶. Primary necrosis refers to caspase-independent, nonapoptotic cell death⁷, whereas secondary necrosis results from inefficient clearance, or ‘efferocytosis’, of apoptotic cells, which then become necrotic⁶. Most work has focused on the relevance and mechanisms of secondary necrosis of lesional macrophages. For example, evidence in humans and animal models has shown that prolonged endoplasmic reticulum (ER) stress can induce advanced lesional macrophage apoptosis⁸ and that efferocytosis of apoptotic macrophages may go awry in advanced atherosclerosis⁶.

But further work is needed to address crucial gaps in this field. Although *in vivo* causation studies have established a role for ER stress in advanced lesional macrophage death⁸, it is not yet known whether molecules known to be present in advanced atheromata and to induce ER stress and apoptosis in cultured macrophages, such as saturated fatty acids or 7-ketocholesterol, are actually responsible for lesional cell death *in vivo*. Altering their accumulation in lesions and then measuring the effect on apoptosis will be needed to address this issue.

Another gap is the molecular basis of defective efferocytosis. One idea is that c-mer proto-oncogene tyrosine kinase (MERTK), a receptor that recognizes and engulfs apoptotic cells and shown to function in early atherosclerotic lesions^{9,10}, becomes disabled in advanced lesions by protease-mediated cleavage, a process shown to occur *in vitro* in response to inflammatory stimuli¹¹. Additional ideas have focused on how other molecules involved in efferocytosis in advanced atherosclerosis might become depleted or dysfunctional⁶. Determining the relevance of these processes to advanced plaques will require additional mechanistic work and new *in vivo* models.

Regarding primary necrosis, *in vitro* studies have shown that macrophages treated with a caspase inhibitor and interferon- γ undergo primary necrosis mediated by the receptor-interacting protein 1 (RIP1)¹². Testing the effect of RIP1 deletion on macrophage death and plaque necrosis in mouse models of advanced atherosclerosis will help identify whether this mechanism leads to primary necrosis in lesions.

Research on the other feature of preculprit lesions, thinning of the collagenous fibrous cap, has focused on the roles of intimal smooth muscle-like cells (SMCs) in collagen production¹³ and macrophage-derived proteases in collagen degradation¹⁴. The death of SMCs in advanced lesions may lead to decreased collagen production, contributing to plaque thinning¹³, but the effect of preventing SMC death on the fibrous cap remains unknown. Mouse models in which matrix metalloproteinases or cathepsins have been deleted or overexpressed have shown effects on plaque collagen or elastin content and integrity, but direct links with plaque rupture and acute thrombosis are not yet possible, as mouse lesions lack these two features¹⁵.

The future challenges related to both lesional necrosis and fibrous cap thinning are substantial and will require development of improved animal models and new imaging techniques in humans¹⁶. Information emerging from these studies will continue to provide a strong foundation for basic research in how plaques progress, which may help identify new therapeutic targets to prevent the deadly minority of atherosclerotic lesions that trigger the leading cause of death worldwide.

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Figure 1.

Features of a culprit atherosclerotic lesion. Lesions that are identified *post hoc* as having caused an acute atherothrombotic event have three key features—a large necrotic core, a ruptured or eroded fibrous cap and an overlying occlusive thrombus. The necrotic core is formed by progressive death of macrophage foam cells, accompanied by defective clearance (efferocytosis) of the dead cells by nearby living macrophages or dendritic cells (DCs). The necrotic core, together with inflammatory macrophages and possibly death of collagen-producing smooth muscle cells, leads to thinning and then rupture or erosion of the fibrous cap. The breach in the cap exposes lesional thrombogenic material to the lumen, which can cause acute, occlusive thrombosis and ischemia or infarction of distal myocardial tissue. Earlier, nonculprit lesions that have two key features—a large necrotic core and thinning of the fibrous cap—have a higher probability of progressing to the culprit stage compared with lesions without these features and thus are termed ‘vulnerable plaques’.