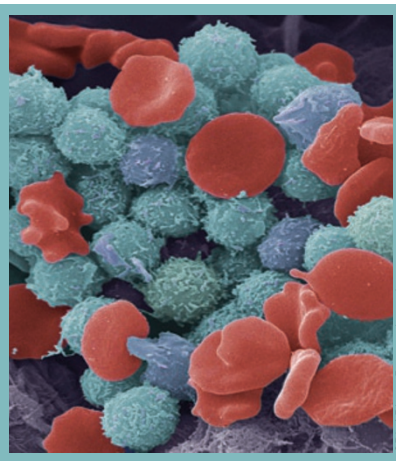
The phantom menace?



Most, if not all, leukemic cells have stem cell–like potential to sustain tumor growth, according to a recent report in *Science* (**317**, 337).

Tumors arise as clones of a single cell, but classic transplant experiments suggested that few malignant cells within an established tumor have the potential to drive its growth. When transferred into immunocompromised mice, only a few human leukemic cells initiate tumors (an estimated 1 in 10^6 cells). This observation led investigators to propose that tumors require rare cancer stem cells for growth, but it did not account for any effects that differences between donor and host might have on tumor growth, such as incompatibility of growth factors and their receptors.

Priscilla Kelley *et al*. tested these microenvironmental influences with a simple and elegant experiment. Instead of transferring human leukemic cells into mice, the authors transferred 10^5 mouse leukemic cells into a histocompatible mouse, then serially diluted this amount to determine the lowest number of cells that had tumor-initiating potential. They found that mice that had received only ten cells developed leukemia. The only difference between the small and large transfers was a correlated time of leukemia onset, which is consistent with multiple cells in the large sample growing independently and simultaneously. This astonishing result indicates that most leukemic cells have tumor-growth sustaining potential and strikes a blow to the stem-cell hypothesis for leukemia.

Kelley *et al.* propose that the rarity of tumors arising from human leukemic cells reflects the small number of cells that can survive and proliferate in the mouse. The xenotransfer experiments may have been undermined by the microenvironment, which may not be optimal for human cell growth. Growth of breast, brain and colon cancers have also been reported to be driven by rare stem cells, and it remains to be seen whether the issues raised by this study apply to these solid tumors, which generate more cellular diversity than leukemias. Regardless, these findings emphasize the need to clear the body of all leukemic cells to prevent cancer recurrence.

—Katherine Stevens

'Multipurpose oxidase' in atherogenesis

Daniel J Rader & Harry Ischiropoulos

A new mechanism of protein carbamylation links inflammation, cardiovascular disease and smoking (pages 1176–1184).

Despite being much-studied and a worldwide scourge, the pathogenesis of atherosclerotic cardiovascular disease remains incompletely understood. Although inflammation and oxidative stress are generally believed to have important roles in this disease[1], the molecular mechanisms by which they promote the formation of plaques within the cardiovascular wall (atherosclerosis) are still unclear. In this issue of *Nature Medicine*, Wang *et al.*[2] reveal a previously undescribed biochemical pathway that involves the enzyme myeloperoxidase (MPO) and that leads to protein carbamylation, suggesting a new predictive test and therapeutic target of cardiovascular disease.

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While studying the denaturation of proteins by urea, Stark *et al.*[3] observed that the urea-generated molecule cyanate (OCN^-) could modify lysine residues, creating ε-carbamyllysine (also called homocitrulline) in a process called carbamylation. This modification alters protein function, probably through the loss of the positive charges on the lysine residues[3]. Individuals with very high blood levels of urea also have high blood levels of carbamylated proteins[4]. With the exception of this uremic-state carbamylation, however, the chemistry responsible for protein carbamylation *in vivo* has remained elusive.

MPO, a heme protein expressed by leukocytes, catalyzes the formation of the antimicrobial agent hypochlorous acid from chloride and hydrogen peroxide[5]. Other small molecules, including tyrosine, hydroxy–amino acids and nitrite, serve as substrates for MPO, and although it is unclear whether these can also have antimicrobial activity, they certainly have profound effects on the immediate protein and lipid environments. The oxidation of tyrosine to a tyrosyl radical facilitates the oxidative cross-linking of proteins, whereas the oxidation of hydroxy–amino acids forms advanced glycation end products[6]. The oxidation of nitrite by MPO leads to reactive nitrogen species that cause the formation of 3-nitrotyrosine, another putative biomarker for cardiovascular disease[7].

In the current study, Wang *et al.*[2] show that thiocyanate (SCN^-) is yet another substrate for MPO, and its oxidation results in the formation of cyanate (OCN^-), which then covalently modifies lysine residues on proteins and lipoproteins to form homocitrulline (**Fig. 1**).

After elegantly revealing the *in vitro* chemistry, the authors went on to demonstrate that this process occurs *in vivo* in mice and in humans[2]. Chemically induced peritonitis led to a dramatic increase of protein-homocitrulline levels, which were substantially reduced in MPO-deficient mice, suggesting that this modification is increased during inflammation and is dependent on MPO. Because atherosclerotic lesions are known to be inflammatory and because increased MPO is a known risk factor for heart disease, the authors then assessed whether this process occurs in such lesions. They found carbamylated proteins and lipoproteins within atherosclerotic lesions in mice as well as in humans. Although this observation did indicate that the carbamylation process occurs in these lesions, it was unclear whether the carbamylated proteins were a cause or an effect of plaque formation.