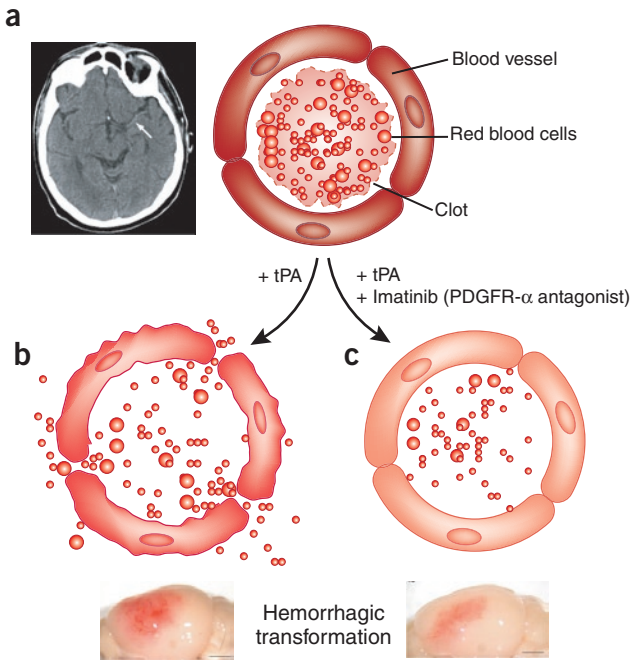

Katie Ris-Vicari

Figure 1 Delayed tPA treatment in thrombembolic stroke. (**a**) Ischemic stroke, as detected by the dense artery cerebri media sign on a computed tomography scan (white arrow), with complete thrombotic occlusion of the vessel. (**b**) At the level of smaller distal arterioles, delayed thrombolytic treatment with tPA may result in increased BBB permeability and eventually hemorrhagic infiltration. (**c**) This severe side effect can sufficiently be blocked by oral application of the PDGFR-α antagonist imatinib. Bottom images, of mouse brain after delayed tPA treatment (5 hours) reprinted with permission from Su *et al.*[7]

When they blocked the pathway downstream of PDGFR-α, the researchers observed substantially reduced cerebrovascular permeability and hemorrhagic complications even after late application of tPA—five hours after occlusion of the middle cerebral artery[7] (**Fig. 1**). Application of imatinib one hour after vessel occlusion and delayed tPA application after five hours resulted in a reduction in infarct volume by 34% compared to tPA treatment alone. Hemorrhagic complications were also reduced by 50%, as measured with hemoglobin staining, which is associated with the ischemic hemisphere.

Blocking the PDGF-CC–PDGFR-α pathway is unlikely to disrupt tPA's fibrinolytic function. Therefore, strategies specifically targeting the PDGF-CC–PDGFR-α pathway should maintain tPA's beneficial thrombolytic activity while minimizing BBB dysfunction. A single high dose of oral imatinib may be sufficient to extend the standard three-hour window for tPA by reducing hemorrhagic complication and restoring neuroprotection.

Exactly how activated PDGF-CC mediates BBB damage is still not clear, but the authors provided evidence that the cell surface receptor low-density lipoprotein receptor–related protein (LRP) is involved in this process[9]. This finding dovetails with previous work showing that the excitotoxic effects of tPA are partly mediated through binding to LRP[6] and cleavage of the NR1 subunit of the *N*-methyl-D-aspartate receptor, which leads to increased calcium influx and subsequent neuronal death[10].

These findings have implications beyond stroke, as endogenous activation of tPA perpetuates the pathogenic process in other conditions such as seizures and neurodegeneration. Although imatinib does not efficiently cross the BBB in healthy individuals, it still manages to get into the brain at detectable levels after oral administration[11]. Longer application periods of imatinib are not desirable because PDGFs seem to promote tissue restoration and angiogenesis after central nervous system injury[12]. Therefore, a one-shot application of imatinib before tPA application in stroke may work to extend the time period for safe thrombolysis and may provide additional neuroprotection.

Ischemic stroke still leaves many of its victims permanently disabled, and the field has suffered from a 'translational' roadblock in taking ideas from the bench and into clinical trial[13]. Fortunately, this roadblock has not proven insurmountable: the first randomized clinical trial to evaluate the safety and feasibility of imatinib for treatment of ischemic stroke, either alone or in combination with tPA, is about to start[7].

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Rejuvenating premature aging

Eran Meshorer & Yosef Gruenbaum

Two commonly prescribed drugs, statins and aminobisphosphonates, may be helpful in combating the rare aging disorder, Hutchinson-Gilford progeria syndrome (pages 769–774).

New findings in this issue of *Nature Medicine*[1] provide insight into a potential treatment for Hutchinson-Gilford progeria syndrome (HGPS), an extremely rare disease in which young children appear to age rapidly. The disease is caused by point mutations in the gene encoding the nuclear structural proteins lamin A and lamin C. As a result, lamin A becomes permanently farnesylated, a modification in which a farnesyl group is added to a protein. Similarly, specific mutations in the lamin A–processing enzyme, ZMPSTE24, cause permanent farnesylation that leads to premature aging in humans.

Drugs that inhibit farnesylation are being tested in clinical trials, but it seems

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that the drugs may open the door to another type of post-translational modification of lamin A, geranylgeranylation, which involves the attachment of a different prenyl group to a protein. The new findings, in a mouse model, suggest that inhibiting both farnesylation and geranylgeranylation may be more effective at restoring normal cellular function and combating the disease (**Fig. 1**).

Children with HGPS suffer from afflictions characteristic of older people—these include growth retardation, hair loss, skeletal alterations, loss of subcutaneous fat, skin atrophy and atherosclerosis. Most people with HGPS die from myocardial heart attack at a mean age of 13.5 years. Cultured fibroblasts from individuals with HGPS show lobulation and invagination of nuclear membranes, thickening of nuclear lamina, loss of peripheral heterochromatin, delayed mitosis and early senescence, which is mediated by the p53 pathway[2]. Nuclear lamins are intermediate filament proteins that assemble into higher order structures and serve as platforms for numerous nuclear proteins required for several nuclear activities. The gene encoding lamins A and C, *LMNA*, is one of the three lamin genes in humans; the others are *LMNB1* and *LMNB2*. Although every cell in the body expresses at least one of the lamin B proteins, lamin A is expressed mostly in differentiated tissues and in some adult stem cells[3,4].

Lamins B1, B2 and A are translated as prelamins with a C-terminal CAAX motif. They are subjected to three post-translational modifications. After the cysteine is farnesylated, the last three residues (AAX) are cleaved by either Ras converting enzyme-1 or the zinc metalloproteinase ZPMSTE24 (also known as FACE-1), and, subsequently, the cysteine undergoes methyl esterification. Lamin A undergoes a fourth maturation step in which the last 15 residues at its C-terminus are cleaved off by ZPMSTE24 (ref. 5).

The most common mutation in people with HGPS is a single nucleotide substitution (C→T at position1824) in the *LMNA* gene. This mutation, which activates a cryptic splice donor, results in a truncated mRNA that is translated into lamin A lacking the second ZPMSTE24 cleavage site. This mutant lamin A, termed progerin or LAΔ50, is permanently carboxyfarnesylated and methylated and is toxic to cells[6]. Similarly, mutations in the human ZMPSTE24 result in permanently carboxyfarnesylated and methylated prelamin A, causing premature aging phenotypes. Mouse models that are either null for *Zmpste24* or with lamin A lacking the second Zmpste24 cleavage site show age-related nuclear lamina defects and progeroid-like symptoms[7,8]. The finding that the accumulation of farnesylated prelamin is toxic to cells[6] has suggested that inhibiting lamin farnesylation with farnesyltransferase inhibitors (FTIs) might be useful in progeria.

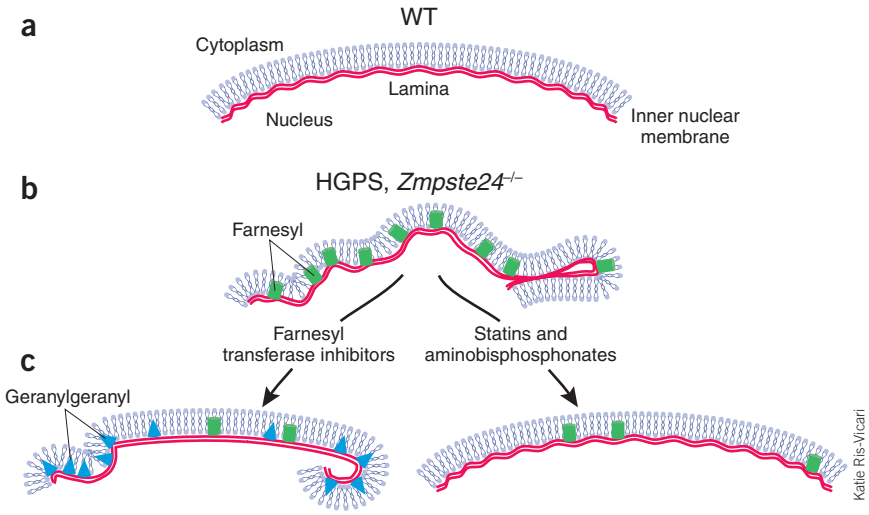

Figure 1 Lamin A modifications in a model of HGPS. (**a**) In wild-type cells, lamin A is farnesylated at its C-terminal CAAX box; cleavage of lamin A by ZMPSTE24 normally removes the farnesylated cysteine residue (top). (**b**) Mutations in ZMPSTE24 cause permanent farnesylation of lamin A, leading to aberrant nuclear morphology and causing progeria (middle). Permanent farnesylation is also observed in HGPS cells and occurs in mouse models of the disease in which ZMPSTE4 is mutated. (**c**) Farnesyl transferase inhibitors reduce lamin A farnesylation but induce an alternative prenylation pathway leading to lamin A geranylgeranylation. Varela *et al.*[1] inhibit both farnesylation and geranylgeranylation by using a combination of statins and aminobisphosphonates; the treatment inhibits both forms of lamin A prenylation, restores nuclear morphology and eases disease-related symptoms.

Previous work has shown that treating HGPS or *Zmpste24*-null cells with FTIs caused displacement of progerin to the nucleoplasm and restored nuclear shape[9]. Treating 5-week-old *Zmpste24*-null mice with FTIs improved their growth, body weight, life span and bone defects[10,11], findings that have led to the first clinical trial of the FTI ionafarnib in children with HGPS.

Varela *et al.*[1] went a step further. They speculated that in the presence of FTIs lamin A may be alternatively prenylated by geranylgeranylation[1], as has been observed for the K-Ras and N-Ras proteins in cells treated with FTIs[12]. Indeed, separate and combined treatments with an FTI and a geranylgeranyltransferase inhibitor (GGTI) showed that both prelamin A and progerin are alternatively geranylgeranylated in the presence of FTIs.

The authors then turned to target an upstream moiety of the lamin A prenylation pathway, farnesyl pyrophosphate, which acts as a substrate of both farnesyltransferase and geranylgeranyltransferase, thus inhibiting both farnesylation and geranylgeranylation of lamin A[1]. They used a combined treatment of statins (pravastatin) and aminobisphosphonates (zoledronate)[1]. These drugs efficiently inhibited the prenylation of prelamin A and progerin in *Zmpste24*-null mice and HGPS cells, respectively, restoring the nonprenylated state of lamin A, nuclear shape and nuclear architecture.

The authors went on to test the effects of combined treatment of pravastatin and zoledronate on *Zmpste24*-null progeroid mice[1]. They found a marked improvement in HGPS-like symptoms, including increased fat deposits and body weight, rescued bone density, reduced curvature of the spine and reduced hair loss. Most importantly, the drug increased life span of the mutant mice by almost 80%[1]. Nuclear morphology was restored and the p53 pathway was deactivated. These effects seem to be superior to treatments with FTIs alone. It remains to be seen whether pravastatin and zoledronate will have similarly beneficial effects in mice expressing progerin/LAΔ50.

This study sheds light on the prenylation mechanism of lamin A, revealing its geranylgeranylation modification in the presence of FTIs. Because statins and aminobisphosphonates are commonly prescribed drugs, it is probably safe to go ahead and test the combination of these on subjects with HGPS.

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Chipping away at gallstones

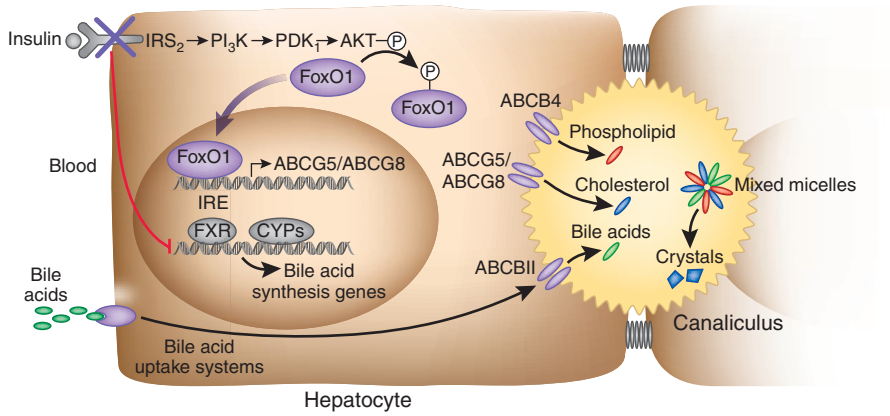
Folkert Kuipers & Albert K Groen

Gallstone disease occurs more frequently in subjects with the metabolic syndrome and type 2 diabetes. Findings in a mouse model suggest that the forkhead transcription factor FoxO1 lies behind this association (pages 778–782).

Age, female gender and obesity are well-known risk factors for cholesterol gallstone disease, which has an incidence of up to 15% in the Western world. The association between gallstones and obesity is likely to reflect an association with the metabolic syndrome, a highly prevalent condition characterized by insulin resistance (insensitivity of target organs and tissues to the actions of insulin), elevated plasma lipid levels, hypertension and increased risk of cardiovascular disease. Insulin resistance can progress to type 2 diabetes, and insulin-resistant subjects with type 2 diabetes have a two- to threefold higher prevalence of gallstone disease[1].

In this issue of *Nature Medicine*, Biddinger *et al.*[2] identify a molecular link between insulin resistance and gallstone formation. They show that the winged helix/forkhead transcription factor FoxO1 helps drive the formation of gallstones in response to hepatic insulin resistance in a mouse model.

Gallstones develop when the liver secretes bile that is supersaturated with cholesterol. Under normal conditions, the water-insoluble cholesterol molecules in bile are solubilized by bile acids and phospholipids (**Fig. 1**). When the cholesterol concentration in bile exceeds the solubilizing capacity of bile acids and phospholipids, supersaturation of bile with cholesterol leads to the formation of cholesterol crystals that can nucleate gallstone growth. Cholesterol gallstones consist of more than 80% pure crystalline cholesterol. They are formed primarily in the gallbladder, where bile is concentrated before it is discharged into the small intestine after a meal to aid the absorption of dietary fat. Phospholipids, cholesterol and bile acids are the three major solids present in bile.



Katie Ris-Vicari

Figure 1 Bile formation by hepatocytes. Transport of bile acids by the ATP-binding cassette transporter ABCB11, found at the canalicular membrane, drives bile flow. Secretion of bile acids also drives the secretion of phospholipids and cholesterol into bile through the ABCB4 and the ABCG5/ABCG8 heterodimer, respectively. Within the bile canalicular lumen, bile acids, phospholipids and cholesterol form mixed micelles and vesicles that keep water-insoluble cholesterol molecules in solution. Cholesterol concentrations that exceed this solubilizing capacity give rise to supersaturated bile containing cholesterol crystals that seed gallstones. On the basis of their studies in LIRKO mice, which have selective insulin resistance in the liver, Biddinger *et al.*[2] propose two mechanisms that underlie predisposition to gallstone formation in individuals with the metabolic syndrome. First, LIRKO mice show reduced formation of bile acids. Second, the absence of hepatic insulin signaling in these mice increases the transcriptional activity of FoxO1, through inhibition of Akt-mediated phosphorylation. As a result, ABCG5/ABCG8 expression is induced.

Bile acids are amphiphatic molecules synthesized in liver from cholesterol by two well-defined pathways[3]. The ATP-binding cassette transporter ABCB11 transports the bile acids across the apical membrane of liver cells into the bile canaliculi, the minute bile channels between adjacent hepatocytes. More than 95% of the secreted bile acids are subsequently reabsorbed from the small intestine and cycle back to the liver for resecretion into bile. In the canalicular space, bile acids drive secretion of phospholipids and cholesterol from liver cells into the bile. The ABC transporter ABCB4 (ref. 4) and the heterodimer ABCG5/ABCG8 (ref. 5) mediate transport of phospholipids and cholesterol, respectively, into the bile.

The intracellular routing of lipids destined for the bile, as well as the activity of ABC transporters, are under coordinated control of numerous nuclear receptors and transcription factors. This complexity has made it difficult to define the mechanisms underlying gallstone formation.

To get at the problem, Biddinger *et al.*[2] used the liver-specific insulin receptor knockout (LIRKO) mouse model. These mice are resistant to the actions of insulin exclusively in the liver,

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