Enhanced eNOS Activation as the Fountain of Youth for Vascular Disease

Is BPIFB4 What Ponce de León Was Looking For?

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Ponce de León was a 16th century Spanish explorer in search of the fountain of youth, a source of magical water capable of reversing the aging process. Whether this tale existed at all, was a fleeting interest of King Ferdinand or a motivational tactic for his crew leading to the eventual discovery of Florida is open for historical speculation. However, by 2020, for the first time in history, people ≥65 years of age will outnumber children (<5 years of age) in the world.[1] As the prevalence of cardiovascular diseases rises with aging independent of other risk factors,[2] identifying genetic variations in centenarians might help to treat or prevent the onset of diseases at earlier stages of life and aid in the discovery of new pathways to promote longevity with less disease.[3,4] Indeed, exceptional longevity is a heritable trait that is associated with less cardiovascular risk compared with younger populations but the genetic basis of cardioprotective mechanisms in centenarians is not yet known.

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Nitric oxide (NO) produced by endothelial NO synthase (eNOS) promotes various beneficial functions in the cardiovascular systems because NO is a potent vasodilator, prosurvival, anti-inflammatory, and antioxidant autacoid. Endothelial dysfunction defined as a reduction in NO bioavailability or responsiveness, is a hallmark of many cardiovascular diseases, including aging. Therefore, therapeutic agents restoring endothelial function to treat cardiovascular diseases, such as hypertension or atherosclerosis are of clinical interest and may affect age-related vascular disease.

In this issue of *Circulation Research*, Villa et al[5] provide striking evidence that a variant (I229V) of BPI fold-containing family B, member 4 (BPIFB4/LPLUNC4) is a gene associated with exceptional longevity. This gene variant and 3 additional associated haplotypes were discovered using strict thresholds of significance for genome-wide association studies in 2 independent cohorts of centenarians in Europe and United States. The first test of the hypothesis examined BPIFB4 in circulating CD34$^+$ cells isolated from long-lived individuals, and BPIFB4 mRNA levels are elevated in long-lived individual samples, and this increase is found both in late outgrowth endothelial cells (from isolated and cultured CD34$^+$ cells) and CD34-positive mononuclear cells. Moreover, eNOS phosphorylation levels on serine 1177,[6] a well-characterized phosphorylation site linked to enhanced eNOS function, were augmented in mononuclear cell extracts of subjects carrying the a/a BPIFB4 allele (but not A/A or A/a alleles) of the nonsynonymous single nucleotide polymorphism, rs2070325. These data led to the hypothesis that BPIFB4 may modulate vascular tone, perhaps by regulating eNOS function. Indeed, BPIFB4 levels are reduced in aged mice and knockdown of BPIFB4 using siRNA reduces vascular function while overexpression of a longevity-associated variant (LAV-BPIFB4), improves age-related endothelial dysfunction in isolated vessels, reduces blood pressure in hypertensive rats, and improves ischemic recovery.

Mechanistically, how does this variant regulate eNOS phosphorylation? Villa et al[5] show that BPIFB4 is a substrate for the enzyme protein kinase R–like endoplasmic reticulum kinase and has an atypical 14-3-3 binding domain. The BPIFB4 variant found in long-lived individuals is preferentially phosphorylated by protein kinase R–like endoplasmic reticulum kinase and phosphorylation restrains BPIFB4 in the cytosol strengthening its interaction with 14-3-3, a scaffolding protein for phosphorylated proteins. The BPIFB4/14-3-3 complex can recruit heat shock protein 90 kDa, a well-known activator of eNOS.[7] Previous work has shown that heat shock protein 90 kDa recruitment to eNOS facilitates eNOS phosphorylation by the protein kinase Akt[8,9] and the data by Villa et al[5] adds another layer of sophistication to control eNOS activity (Figure).

As with any new discovery, there are many interesting questions to be explored. Do patients harboring the rs2070325 single nucleotide polymorphism have normal endothelial function and less vascular disease? This should be testable given the number of patients with this genotype. How do the levels of BPIFB4 in CD34$^+$ cells relate to potential benefit in long-lived individuals? Assuming that these cells are potential surrogates, are the levels of BPIFB4 and eNOS phosphorylation elevated in endothelium lining conduit and resistance arteries? Because both wild-type and LAV-BPIFB4 induce the adaptive stress response, how does the LAV allele afford unique activation of eNOS compared with wild-type BPIFB4? Finally, given that eNOS is regulated by a variety of

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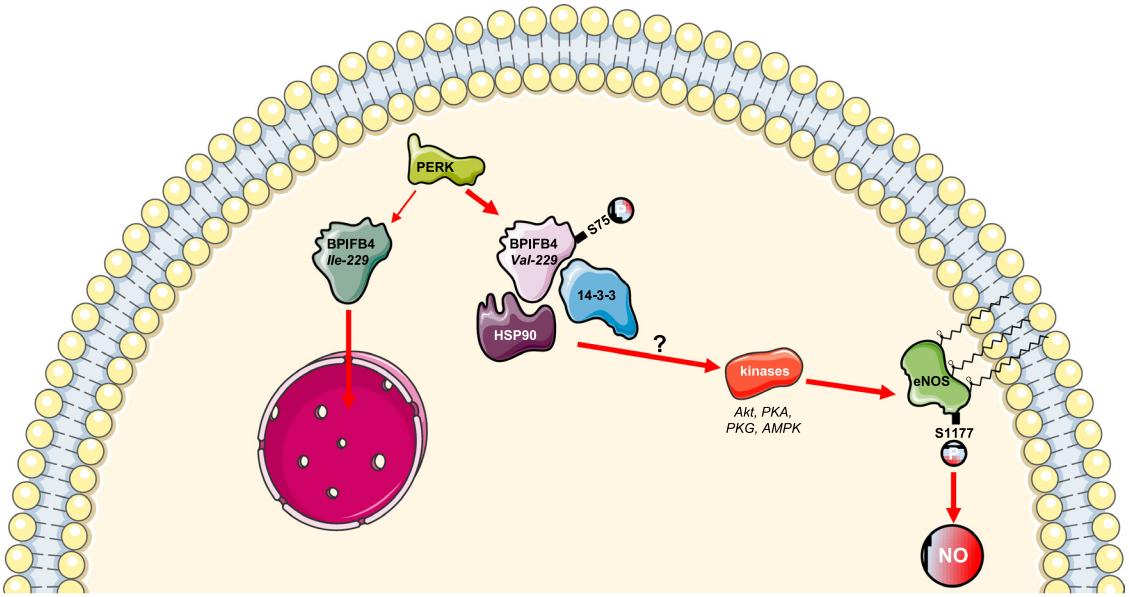

Figure. PERK/BPIFB4/eNOS pathway. Protein kinase R–like endoplasmic reticulum kinase (PERK) preferentially phosphorylates BPI fold-containing family B, member 4 (BPIFB4)-V229 on serine-75. Phosphorylated BPIFB4 is retained in the cytosol by binding to 14-3-3 and subsequentially binds to heat shock protein 90 kDa (HSP90). This complex leads to phosphorylation of endothelial NO synthase (eNOS) by several potential kinases on serine-1177, a site linked to enhanced eNOS function. Akt, also called protein kinase B (PKB); AMPK indicates AMP-activated protein kinase; NO, nitric oxide; PKA, protein kinase A; and PKG, protein kinase G.

phosphorylation events and protein–protein interactions, how does BPIFB4 fit into the fold? Given the potential contribution of BPIFB4 to protecting the cardiovascular system during aging, Ponce de León would be interested and perhaps envious of the discovery of BPIFB4 promoting longevity-associated endothelial function and health.

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Disclosures

None.

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