In patients with degenerative mitral valve disease without intrinsic lung parenchymal disease, pulmonary hypertension regression one year following mitral valve repair occurs less often in older patients, and in those with preoperative chronic atrial fibrillation or preoperative left ventricle dysfunction. Persistent pulmonary hypertension may exist even with low postoperative mean postoperative mitral gradients.

**METHODS:** EA.hy926 endothelial cells were treated with ER stress-inducing agent Thapsigargin (Tg) with or without APC (10, 30, 50 nM) for 1 hour and up to 18 hours. Western blotting technique was performed to study the expression status of ER stress markers (p-eIF2α, BiP, ATF6, p-IRE1) and the pro-apoptotic protein (CHOP). Following 1 hour of treatment. Adding APC at a dose of 50 nM to this dose of Tg significantly dampened the excess phosphorylation of p-eIF2α; however it didn’t influence the increased expression of BiP, which occurred with Tg treatment. Tg did not activate p-IRE1 or ATF6 in our experiments. After 18 hours of treatment with Tg, CHOP protein was induced in the endothelial cells suggesting ER stress-induced apoptosis, which was also abrogated by APC treatment.

**CONCLUSION:** APC suppresses the p-eIF2α pathway of ER stress in endothelial cells and therefore it alleviates the protein synthesis inhibition caused by increased phosphorylation of p-eIF2α. This effect of APC appears to protect the endothelial cells from apoptosis by inhibiting the expression of CHOP downstream of p-eIF2α. Altogether, these results indicate that some of the anti-apoptotic and anti-inflammatory effects of endogenous APC may be mediated by the alleviation of ER stress in the endothelium.

**RESULTS:** Tg(100 nM) caused significant up-regulation of ER stress markers (p-eIF2α, BiP) after 1 hour of treatment. Adding APC at a dose of 50 nM to this dose of Tg significantly dampened the excess phosphorylation of p-eIF2α; however it didn’t influence the increased expression of BiP, which occurred with Tg treatment. Tg did not activate p-IRE1 or ATF6 in our experiments. After 18 hours of treatment with Tg, CHOP protein was induced in the endothelial cells suggesting ER stress-induced apoptosis, which was also abrogated by APC treatment.

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**BACKGROUND:** Inhibition of Four-and-a-half LIM domain protein-2 (FHL-2) attenuates atherosclerotic lesion formation and increases endothelial cell migration. Endothelial progenitor cells (EPCs) substantially contribute to endothelial repair. We investigated the role of FHL-2 in the regulation of early outgrowth EPC number and function.

**METHODS AND RESULTS:** Early outgrowth EPCs were obtained from human peripheral blood. FHL-2 knockdown in EPCs by small-interfering RNA (siRNA) resulted in a significant increase in EPC number and a reduction of apoptosis (by 40%), as indicated by a decrease of cleaved caspase-3, through activation and translocation to the membrane, of sphingosine kinase-1 (SK-1), enzyme that metabolizes sphingosine-1-phosphate (s1p). Furthermore, FHL-2 siRNA increased significantly (2 fold) stromal derived factor (SDF) -1- induced EPC migration; through upregulation of α-v/β-3 and α-v/β-5 integrins; this was associated with an increase of the F-actin binding protein cortactin, known to promote migration. Interestingly, increased SDF-1- induced EPC migration and up-regulation of cortactin by FHL-2 siRNA were totally prevented by CAY10621, a specific inhibitor of SK-1. In addition stimulation of EPCs with exogenous s1p peptide significantly decreased apoptosis and increased SDF-1- induced migration. These results were confirmed In vivo using FHL-2 knockout (FHL-2 −/−) mice. Moreover, apoptosis was significantly decreased and migration increased in endothelial cells exposed to the conditioned medium of FHL-2 −/− vs. WT EPCs. These effects were abolished by VPC23019, an antagonist of sphingosine-1-phosphate receptor-1 and 3. Finally, reendothelialization after focal carotid endothelial electric injury in WT mice was significantly increased after application of spleen-derived progenitor cells from FHL-2 −/− mice vs. WT mice.

**CONCLUSION:** Our findings suggest that FHL-2 negatively regulates early outgrowth EPC function and secretion of paracrine factors. FHL-2 inhibition reduces apoptosis, enhances survival and migratory capacity of EPCs and ECs by upregulating SK-1/s1p pathway, integrin subunits and cortactin; which results in the improvement of endothelial regeneration.