Hyperhomocysteinemia and Occlusive Vascular Disease

An Emergent Role for Fibroblast Growth Factor 2

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Occlusive vascular disease is the leading cause of mortality and disability in the Western world. Based in accumulated previous evidence, in 1991 Clarke et al identified hyperhomocysteinemia as a new independent risk factor for vascular disease. Since then, hyperhomocysteinemia has been associated with an increased risk of cardiovascular disease, including atherosclerosis, thrombosis, stroke, and peripheral arterial occlusive disease. However, there is no clear consensus as to the potential mechanisms, whereby excess homocysteine could contribute to vascular disease, although endothelial cell damage and/or injury should be expected. In the absence of a clear mechanism linking homocysteine to cardiovascular disease, controversy remains concerning the actual role of hyperhomocysteinemia as an independent or a conditional risk factor or simply a marker of cardiovascular disease. In the present issue of Circulation Research, Chang et al show that a specific homocysteine-induced downregulation of fibroblast growth factor (FGF2) disrupts endothelial integrity by both decreasing endothelial cell proliferation and inducing endothelial cell apoptosis. Recently, it has been claimed that new studies providing insight into the regulatory effects of elevated homocysteine levels are crucial for the development of new diagnostic and therapeutic methods. In this sense, the report by Chang et al adds relevant and valuable information, unveiling FGF2 gene expression as a specific molecular target of homocysteine at pathophysiological levels.

At the molecular level, several potential mechanisms have been proposed previously, including induction of endoplasmic reticulum stress and unfolded protein response (UPR), protein N-homocysteinylaitation, and epigenetic effects concerning methylation status. Until now, the connection of homocysteine with the UPR seemed specially relevant because of a number of observations, including: (1) in human endothelial cells, homocysteine activates the UPR, which is an important regulator of inflammatory genes in these cells; (2) UPR activation by homocysteine induces endothelial cell apoptosis; and (3) homocysteine induces vascular endothelial growth factor (VEGF) expression by a mechanism involving UPR in retinal pigmented epithelial cells. The observation on the induction of VEGF is controversial because other authors have shown that homocysteine inhibits VEGF protein expression in mouse brain microvascular endothelial cells. Furthermore, homocysteine has been shown to induce angiostasis by impaired angiogenic response involving endothelial cell proliferation, migration, and tube formation. In their work, Chang et al show that only high, nonphysiological concentrations of homocysteine (0.5 mmol/L) are able to induce changes in the expression levels of VEGF and that these changes are in agreement with the inhibition previously shown in mouse microvascular endothelial cells and in contrast to the inducing effect reported in retinal pigmented epithelial cells.

On the other hand, because homocysteine is a redox active compound, induction of oxidative stress is among the most favored postulated mechanisms of homocysteine toxicity. On the contrary, potential protective effects of homocysteine have also been reported. It has been shown that extracellular superoxide dismutase, an important antioxidant in vascular tissues, is positively associated with homocysteine levels. At least under certain conditions, homocysteine behaves as an antioxidant. Furthermore, homocysteine reduces the expression levels of endothelin-1, a well-known potent vasoconstrictor. A potent modulatory effect of homocysteine on plasma membrane electron transport systems has also been recently reported. The recent observation that hyperhomocysteinemia inhibits reverse cholesterol transport by reducing circulating HDL via inhibiting the antioxidant apolipoprotein A-I protein synthesis and enhancing HDL cholesterol clearance seems specially relevant. Concerning cholesterol circulation, the group of the present report had previously demonstrated that copper-oxidized and an electro-negative LDL isolated from hypercholesterolemic or type 2 diabetic human plasma can inhibit endothelial cell proliferation and angiogenesis. These angiostatic effects were accompanied by FGF2 downregulation and reversed by FGF2 supplementation. Because the ability of these modified LDLs to inhibit endothelial cell proliferation and angiogenesis resembles that of homocysteine previously shown, Chang et al decided to examine whether homocysteine can modulate FGF2 expression. This is, in fact, the case because, in human coronary and bovine aortic endothelial cells, pathophysiological concentrations of homocysteine exerted time- and concentration-dependent reduction of both the mRNA and protein levels of FGF2. This study clearly shows that FGF2 is, indeed, much more susceptible to homocysteine than VEGF in terms of gene expression, because FGF2 is suppressed with 100 μmol/L homocysteine, and VEGF, in contrast, is only reduced from 500 μmol/L homocysteine.
Furthermore, Chang et al.\(^6\) dissec the effect of homocysteine on FGF2 expression: (1) they observe that this downregulation of FGF2 is specifically attenuated by inhibiting G protein biosignaling; (2) reporter gene assays show that homocysteine behaves as a transcriptional repressor of the gene promoter; (3) an analysis of the human FGF2 gene identifies an 1877-bp CpG island spanning from −532 in the 5′-flanking region and extending through exon 1 into the first intron; and (4) in spite of decreased methylation potential (as revealed by 5-adenosylmethionine to 5'-adenosylhomocysteine ratio values), this CpG island was heavily methylated at the cytosine residues in treatments with pathophysiological concentrations of homocysteine. Therefore, the transcriptional repression of FGF2 expression by homocysteine seems to be mediated by an epigenetics process that alters promoter DNA methylation pattern.

In conclusion, the relevant contribution by Chang et al.\(^6\) adds new insights to the involvement of hyperhomocysteinemia with occlusive vascular disease, identifies repression of the angiogenic factor FGF2 as a new effect of hyperhomocysteinemia, and could have important clinical implications because of the key physiopathological roles of angiogenesis. Furthermore, because homocysteine has also been shown to inhibit the invasive potential of cancer cells,\(^{29}\) understanding the mechanisms by which homocysteine can modulate angiogenesis may suggest novel therapeutic strategies for cardiovascular disease and cancer.

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None.

**References**


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