Insulin Resistance Affects Gene Expression in Endothelium

To the editor:

We and others have found that either genetic insulin resistance or hyperinsulinemia may dampen insulin ability to activate the PI3-kinase/Akt pathway, leading to perturbation of endothelial nitric oxide synthase (eNOS) activation.1,2 However, little is known about insulin effects on transcription of antiatherogenic and proatherogenic genes in insulin resistant conditions. Therefore, we used a genetic model of vascular insulin resistance, primary human umbilical vein endothelial cells (HUVECs) naturally carrying the G972R Insulin Receptor Substrate-1 (IRS-1) variant,3 to investigate the effect of both genetic insulin resistance and hyperinsulinemia on transcription of atherosclerosis-related genes. G972R IRS-1 variant reduces IRS-1 activation of the PI3-K/Akt pathway,3 and it has been associated to coronary artery disease and obesity.3,4 HUVECs carrying the wild-type (WT) or G972R IRS-1 variant were obtained as previously described.3 For the experiments, 3rd through 5th passage HUVEC-WT and 972 were incubated in serum free EGM-2 medium for 16 hours in the presence or absence of insulin 5×10⁻⁷ mol/L, to obtain a full effect of insulin on nitric oxide production.3 Total RNA was extracted from the HUVECs with Trizol reagent according to the manufacturer’s protocol. To profile gene expression pattern we made use of U133A Affymetrix DNA microarray containing a total number of 22283 probe sets corresponding to about 15 000 genes, using previously described methods.6 Gene expression profiling was obtained by comparing HUVEC-WT versus HUVEC-972, both cultured in the presence or in the absence of hyperinsulinemia. Most significantly upregulated or downregulated genes in stimulated HUVEC-972 compared with untreated HUVEC-972 are shown in the Table. Expression of these genes did not differ in untreated HUVEC-WT as compared with untreated HUVEC-972. The major effect of the combination of the genetic variant plus hyperinsulinemia was the upregulation of genes involved in coagulation and fibrinolysis processes among which we found (Table): collagen type I alpha 1 (COL1A1), a major component of fibrous cap in atherosclerotic plaque and which is involved in platelet activation;7 tissue factor pathway inhibitor 2 (TFPI-2), which is involved in the downstream proteolytic mechanisms occurring in the atherosclerotic lesion,8 and thrombomodulin (TM), an anticoagulatory factor related to the extent of vascular disease.9 Among downregulated genes (Table) we found urokinase plasminogen activator (u-PA), an important component of the plasminogen/plasmin system. Downregulation of u-PA could have a prothrombotic role and impair revascularization in damaged tissue.10 To validate microarray results, we performed quantitative real-time polymerase chain reaction (PCR) of most significant genes chosen either for absolute expression or involvement in the pathogenesis of atherothrombosis. Consistent with microarray results, expression of these genes did not differ in unstimulated WT-HUVECs as compared with unstimulated 972-HUVECs. On insulin stimulation HUVEC 972, compared with WT, show a statistically significant increase in the expression of COL3A, TFFI-2, and TM (P<0.01, P<0.001, and P<0.001, respectively; Figure, A) and a statistically significant decrease in the expression of u-PA (P<0.05; Figure, B). Because the genes regulated by G972R variant in HUVECs are involved in prothrombotic and proinflammatory mechanisms, we analyzed the expression of the same genes in carotid plaques from patients who underwent elective carotid surgery (stenosis >70%), WT for IRS1 (n=3, age=73±3, % of stenosis=83±7, fasting glucose=92±4 mg/dL, fasting insulin=33±12 μU/mL), or carrying the G972R IRS-1 variant (n=3, age=73±4, % of stenosis=90±3, fasting glucose=86±8 mg/dL, fasting insulin=33±12 μU/mL). None of them had history of diabetes, to exclude the potential effects of glucotoxicity, although obese patients carrying G972R IRS-1 variant develop manifest hyperinsulinemia, regardless of the fasting levels. Carotid plaques from patients carrying the G972R variant showed enhanced expression of COL3A, TFFI-2, and TM (P<0.001, G972R versus WT for all; Figure, A), and reduced expression of u-PA (P<0.001; Figure, B). To test the validity of these sets of data we also analyzed the expression of genes involved in atherosclerosis process, which were not identified as deregulated in the microarray experiments. Expression of MCP-1 and plasminogen activator inhibitor (PAI)-1 did not differ in HUVECs carrying the G972R IRS-1 variant as compared with those carrying the WT IRS-1 (Figure, C). Differences found in carotid plaques from patients carrying the G972R IRS-1 variant compared with WT IRS-1 likely reflect the involvement of other cell types in atherosclerotic lesions (ie, smooth muscle cells, monocytes/macrophages, lymphocytes). VCAM1 resulted markedly increased in plaque G972R carriers, possibly as a consequence of a different activation state of endothelial cells in vivo with respect to the culture.

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conditions.

Among the various genes analyzed in endothelial cells and carotid plaques, we found an increased expression of prothrombotic genes and a decreased expression of fibrinolytic genes in nondiabetic carriers of the G972R IRS-1 variant. Recently, Berria et al reported an increased content of collagen type III in the extracellular matrix of muscles from insulin resistant obese subjects, which together with our data suggests a role for altered matrix composition in insulin resistance. TFPI-2 could play roles in the regulation of ECM turnover and cell migration/proliferation pathways, favoring plaque vulnerability, our data suggest that the combination of genetic insulin resistance and hyperinsulinemia can result in a possible imbalance of prothrombotic and fibrinolytic genes which on the long term may impact on both the stability and vulnerability of atherosclerotic plaques. However, expression of other genes relevant in the atherosclerosis process, such as MCP1 and PAI1, does not seem to be affected by the presence of the G972R variant under hyperinsulinemic conditions. Because of the complexity of the composition of atherosclerotic plaques which contains several cell types contributing to the production of factors favoring plaque vulnerability, our data are clearly limited.

Nevertheless, we have identified new targets which might help to explain the unbalance between the prothrombotic and fibrinolytic system in hyperinsulinemic insulin resistant patients affected by atherosclerosis.

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Figure. A, Collagen Type III (Col3A), TFPI2, and thrombomodulin (TM) are upregulated in HUVEC-972 cells (black). B, u-PA is downregulated in HUVEC-972 (black bars). C, mRNA Levels of Col3A, TFPI2, TM, and u-PA in carotid artery atherosclerotic plaques from WT and G972R IRS-1 variant carriers. D, Expression of other genes involved in atherosclerosis. *P<0.05, **P<0.01, ***P<0.001 vs WT. Statistical analysis were performed using the Student t test as indicated. All values are represented as mean±SD n=3 for each gene.

Disclosures

None.

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