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Targeting CITED2 for Angiogenesis in Obesity and Insulin Resistance

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Obese patients who have diabetes have higher cardiovascular disease (CVD) morbidity and mortality than individuals without diabetes, and about 70% of mortality in patients with diabetes is caused by CVD events, including coronary heart disease (CHD), hypertension, heart failure, and stroke (1). Data from the Framingham Heart Study (FHS) show that individuals with diabetes have double the CVD risk in men and triple the risk in women after adjusting age, smoking, hypercholesterolemia, and hypertension (2). Furthermore, experimental and clinical studies have found that endothelial dysfunction may play a key role in the impairment of arterial relaxation and the promotion of thrombosis and neointimal proliferation and that these abnormalities are characteristic features of atherosclerosis and associated CHD in patients with diabetes (3). With the progression of atherosclerosis, coronary arteries become obstructed and hypoxia and ischemia occurs (1,3). In response to myocardial ischemia and hypoxia, angiogenic factors including nitric oxide (NO) and vascular endothelial growth factor (VEGF) are mobilized and activated (1,3). VEGF promotes endothelial cell proliferation via its receptor VEGF receptor 2 and stimulates endothelial NO synthase (eNOS) activation for NO production via VEGF receptor 1 (4). Other factors such as angiopoietin-1/2, platelet-derived growth factor BB, matrix metalloproteinases, and fibroblast growth factor also participate in angiogenesis and the maturation of blood vessels (4).

Hypoxia inducible factor 1 (HIF1) is one of the major transcription factors governing hypoxic responses, and it promotes angiogenesis in the setting of myocardial ischemia (5). Normally HIF1 is degraded through a proteasome-mediated pathway under normoxia (6). Under hypoxic conditions, HIF1 is stabilized, translocated to the nucleus, and dimerized to form transcriptionally active complexes that bind to regulatory elements of hypoxia-responsive genes and recruit the transcriptional coactivators/histone acetyltransferases CREB-binding protein (CBP) and E1A binding protein p300 (p300) (6) (Fig. 1). CBP/p300-interacting transactivator with ED-rich tail 2 (CITED2) is a protein with ED-rich tail and has a high binding affinity to the CH1 domain of CBP/p300 and consequently blocks the access of many transcription factors to the CH1 domain including HIF1. On one hand, CITED2 links hormonal signaling to acetylation of peroxisome proliferator–activated receptor γ coactivator 1 α (PGC-1α) in the regulation of gluconeogenesis (7). On the other hand, CITED2 represses HIF1 transcriptional activation, resulting in inhibition of several angiogenic factor expressions, including VEGF, eNOS, and NO production, and consequently in impaired neovascular response to ischemia in insulin-resistant states, such as obesity and type 2 diabetes (8) (Fig. 1). To this point, VEGF is one of the most specific and critical regulators of angiogenesis, and it stimulates endothelial proliferation, permeability, survival, and capillary formation (4). Several insulin-sensitive signal transduction pathways, including phosphoinositide 3-kinase, mitogen-activated protein kinases, and glycogen synthase kinase 3, have been implicated in regulation of HIF1 protein expression and stability (9).

CHD and peripheral vascular disease are complications associated with insulin resistance where neovascularization ability is diminished in the ischemic tissues and organs including myocardium and lower limbs (4). In this issue of *Diabetes*, Wang et al. (10) investigated how insulin regulates CITED2 expression in endothelial cells and CITED2 expression in obese mouse models and patients with insulin resistance and type 2 diabetes. A total of 10 novel genes were identified to be involved in this insulin regulation, and 3 of them (*Adm*, *Cited2*, and *Ctgf)* were downregulated in endothelial cells through a forkhead box O1 (FoxO1) signaling pathway that may modulate angiogenesis in the context of insulin resistance. For example, CITED2 was downregulated

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See accompanying article, p. 3680.

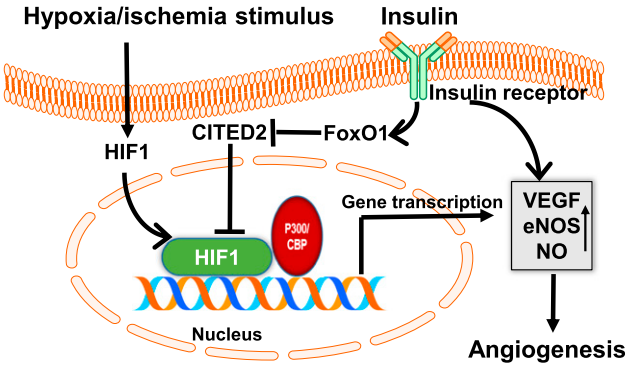

Figure 1—Interaction of CITED2 and HIF1 in the regulation of hypoxia/ischemia-induced angiogenesis in insulin resistance and type 2 diabetes.

by insulin by up to 54% in the cultured endothelial cells, whereas CITED2 in cardiac endothelial cells was increased in both diet-induced obese mice and *db/db* mice. Furthermore, expression of CITED2 in arterial tissues was 3.8-fold higher in patients with type 2 diabetes than in control subjects without diabetes. Although CITED2 overexpression impaired HIF activity in vitro, CITED2 knockdown promoted endothelial tube formation, endothelial cell proliferation, and angiogenesis. Meanwhile, femoral artery ligation in mice prompted expression of HIF1 in hind limb muscle in association with reduced CITED2 in mouse endothelial cells. Therefore, CITED2 may limit HIF1 transcriptional activation and negatively regulate endothelial cell tube formation and angiogenesis in vascular insulin resistance in type 2 diabetes.

The study by Wang et al. (10) provides novel insights regarding interaction of CITED2 and HIF1 signaling in the regulation of endothelial function and angiogenesis in patients with diabetes. This is translationally relevant as therapeutic targeting of CITED2 has a potential application for the prevention of the impaired neovascular responses to ischemia in CHD and peripheral vascular disease in states of insulin resistance. Although these data highlight a central role of CITED2 in the regulation of angiogenesis, several caveats need to be considered. First, CITED2 is an essential transcription factor for normal heart development (11). *Cited2*-deficient mice have been found to show cardiac malformations, adrenal agenesis, and neural crest defects (11). Second, targeting CITED2 to promote angiogenesis may be viewed as a "bad" event in some circumstances. For example, excessive angiogenesis can participate in various human diseases, including cancer, diabetic nephropathy, and diabetic retinopathy (12). Enhanced HIF1 signaling is also regarded as contributing to local inflammation and atherosclerosis (8). Meanwhile, angiogenesis also induces expansion of adipose tissue mass in diet-induced obesity by the sprouting of endothelial cells from preexisting blood vessels (13). Therefore, future studies need to consider CITED2 as a highly selective therapy agent only in "local ischemic revascularization" without undesired consequences.

In conclusion, data in the study by Wang et al. (10) defines a group of endothelial genes, especially *Cited2*, that may be dysregulated in insulin resistance and type 2 diabetes. The interaction of CITED2 and HIF1 impairs endothelial function and angiogenesis during the development and progression of CHD and peripheral vascular disease. These findings indicate that inhibition of CITED2 may be a potential novel therapeutic strategy to improve angiogenesis in ischemic tissues. Further studies are warranted to more definitively define the role of CITED2 in the regulation of angiogenesis and to establish new targeted therapeutic roles for reducing vascular CITED expression in patients with obesity, insulin resistance, and type 2 diabetes.

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