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## CELL BIOLOGY

# Inhibiting the Response to VEGF in Diabetes

Junji Moriya and Napoleone Ferrara\*

**Inhibition of the vascular endothelial growth factor (VEGF)–VEGF receptor 2 (VEGFR2) signaling axis may play a role in endothelial dysfunction and serious vascular complications associated with diabetes. In this issue, Warren *et al.* identified a ligand-independent, receptor tyrosine kinase-independent VEGFR2 signaling pathway that is responsible for impaired responses to VEGF in diabetic endothelial cells. Reactive oxygen species (ROS) resulting from the hyperglycemic status promoted activation and subsequent degradation of VEGFR2 in a ligand-independent manner. Consequently, VEGF-VEGFR2 signaling was inhibited due to depletion of VEGFR2 at the cell surface. Activation of this ligand-independent, ROS-induced VEGFR2 signaling was mediated by the Src family of kinases and occurred in the Golgi compartment in the endothelial cells. Blocking ROS production by antioxidants effectively reversed VEGFR2 deficiency at the cell surface in hyperglycemia. These findings suggest that ROS-induced VEGFR2 signaling might be a promising new target for the treatment of vascular diseases in diabetes.**

Vascular complications associated with diabetes have become serious health problems in the modern world and cause increasing morbidity and mortality. Abnormal angiogenesis plays a major role in the pathogenesis of these conditions (1, 2). In diabetes, angiogenic responses are enhanced in some tissues, such as the retina or kidney, whereas they are impaired in other tissues, resulting in defective wound healing, peripheral circulation, and coronary circulation. Vascular endothelial growth factor (VEGF) has been recognized as the key regulator of both physiological and pathological angiogenesis (3). Several lines of evidence suggest that the VEGF–VEGF receptor 2 (VEGFR2) signaling axis is at least partially inhibited in diabetes (4–6). The study by Warren *et al.* (7) provides new mechanistic insights through investigation of VEGFR2 signaling in endothelial cells. They identified a “noncanonical” VEGFR2 signaling pathway, which is activated in the absence of ligand in the Golgi compartment. Moreover, they found that excessive reactive oxygen species (ROS) produced by hyperglycemia promoted activation of this noncanonical pathway, leading to increased degradation of VEGFR2 in endothelial cells. As a result, “canonical,” ligand-dependent VEGFR2 signaling was inhibited due to decreased availability of

cell-surface VEGFR2 (Fig. 1).

Increased production of ROS is thought to be a key mechanism responsible for vascular damage in diabetic patients. Although low concentrations of ROS contribute to activating canonical, proangiogenic VEGFR2 signaling under physiological conditions, higher concentrations of ROS can cause endothelial damage and impaired angiogenesis. The study by Warren *et al.* shows that chronic ROS exposure activates noncanonical VEGFR2 signaling. By using mice that are selectively VEGF-deficient in endothelial cells, they found that VEGF is not required for activation of this noncanonical pathway. They also showed that ROS-induced phosphorylation of VEGFR2 is VEGFR2-kinase-independent. Because VEGFR2 phosphorylation by ROS is thought to be mediated by VEGF-dependent canonical signaling (8, 9), the involvement of other mechanisms for VEGF-independent, ROS-mediated activation of intracellular VEGFR2 is a new concept.

Another important finding in the study by Warren *et al.* is that ROS-induced phosphorylation of VEGFR2 occurs within the Golgi compartment. There is growing evidence that the Golgi compartment not only processes proteins made in the endoplasmic reticulum but also functions as an activation site for some signaling proteins. The finding that this noncanonical VEGFR2 signaling is mediated by Src family kinases (which is distinct from the canonical signaling) further suggests that the two signaling pathways have different roles in endothelial cells.

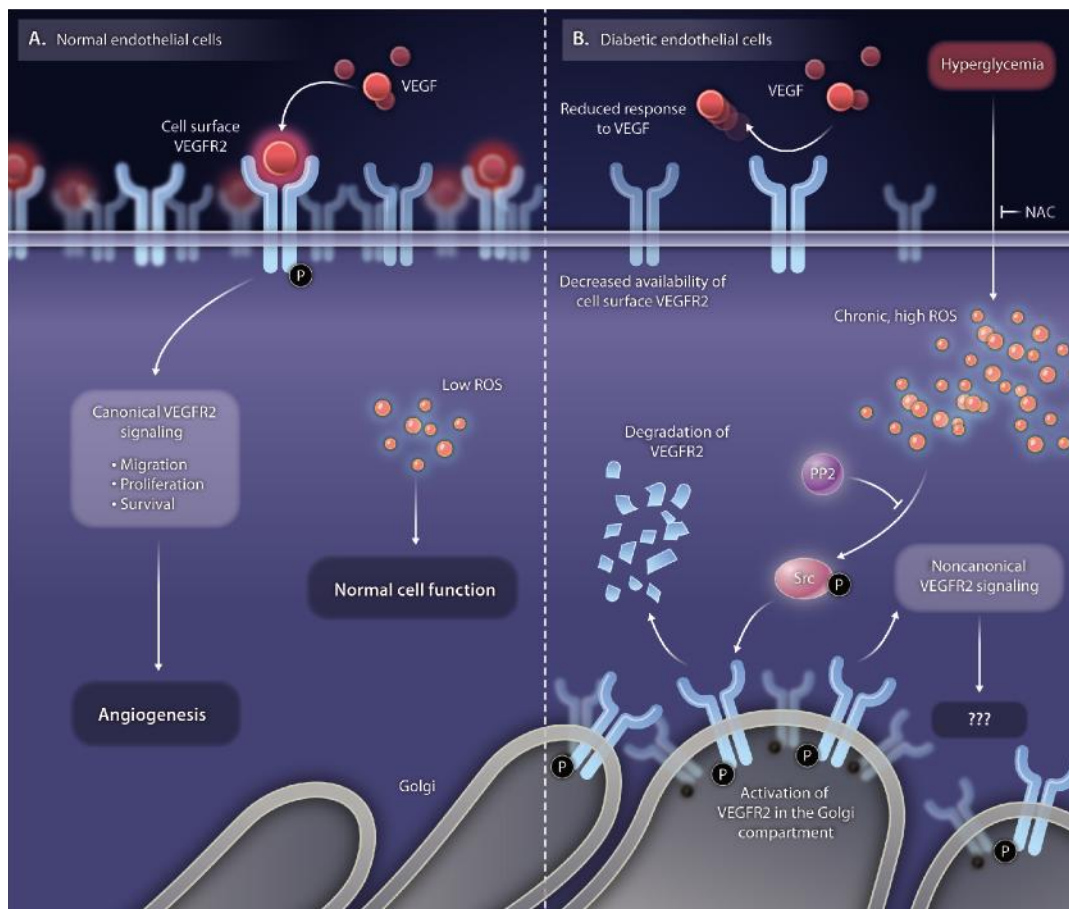
In addition, it is intriguing that depletion of VEGFR2 protein due to excessive activation of VEGFR2 signaling in the Golgi compartment leads to decreased cell surface abundance of VEGFR2, thereby attenuating VEGF-induced angiogenic response. Cellular internalization of growth factor receptors, including VEGFR2, occurs after they have been activated by specific ligands. In many cases, this process is responsible for terminating signaling through degradation of the activated receptor complex. Thus, internalization and subsequent degradation of the activated receptor is now considered an important mechanism by which endothelial cells can regulate the intensity of the signaling (10). The findings by Warren *et al.* (7) confirm that this degradation process occurs within the Golgi compartment and, furthermore, is ligand-independent. It remains to be established whether other receptor tyrosine kinases (RTKs) also have similar ligand-independent signaling that bypasses and inhibits canonical, ligand-dependent signaling.

The difference between canonical and noncanonical VEGFR2 signaling remains an open question. Warren *et al.* show that the noncanonical signaling pathway could compete with the canonical signaling pathway by exhausting VEGFR2 protein. However, precise roles for the noncanonical signaling are yet to be determined, and one can speculate that the noncanonical signaling plays roles distinct from the canonical signaling. Indeed, phosphorylation of p38, a target of VEGFR2, is not likely to occur through Src-mediated noncanonical signaling, whereas both canonical and noncanonical signaling triggered phosphorylation of PLC $\gamma$ , another target of VEGFR2, as reported by Warren *et al.* Moreover, they showed that Tie2, another RTK, is also activated by ROS through Src but that this activation does not lead to reduction in Tie2 protein as it does with VEGFR2. Not only does ROS-induced activation of RTKs through Src seem to be distinct from the canonical, ligand-dependent signaling, its function is also likely to differ depending on individual RTKs. Elucidating the mechanisms of differential effects of ROS-induced, Src-mediated signaling on RTKs remains to be determined.

Finally, the study by Warren *et al.* raises the possibility that antioxidants could rescue impaired response to VEGF in diabetes. They found that treatment with *N*-acetyl-L-cysteine (NAC) substantially increased phosphoryla-

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**Fig. 1. Schematic representation of VEGFR2 signaling in diabetes.** (A) In normal endothelial cells, pro-angiogenic signals triggered by VEGF are conveyed by VEGFR2 at the cell surface (canonical VEGFR2 signaling), and low concentrations of ROS produced by the signaling can act as secondary messengers modulating physiological cell function. (B) In diabetic endothelial cells, hyperglycemia causes excessive, chronic ROS that lead to activation of Src family kinases and subsequent phosphorylation of intracellular VEGFR2. These processes occur within the Golgi compartment and do not require VEGF (noncanonical VEGFR2 signaling). Rapid degradation of the VEGFR2, followed by continuous activation of this signaling, results in decreased availability of VEGFR2 at the cell surface, thereby attenuating responses to exogenous VEGF.

tion of VEGFR2 in response to VEGF both in vitro and in vivo. The NAC-induced amelioration was due to restoration of VEGFR2 in the cell surface by blocking ROS and subsequent phosphorylation of VEGFR2 in the noncanonical pathway. Thus, inhibition of ROS or other events leading to suppression of this noncanonical signaling might be a promising therapeutic strategy for patients suffering from diabetic vascular complications.

In conclusion, the study by Warren *et al.* elucidates an intriguing mechanism of how VEGF-induced angiogenesis is attenuated in diabetes. Identification of this new mode of VEGFR2 activation in endothelial cells might lead to a better understanding of pathogenesis of impaired angiogenesis in diabetes. Similar strategies could be used in the future to investigate other angiogenesis-related RTKs, which might also have implications for the pathophysiology of diabetes.

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