Letter to the editor

Nitric oxide and angiogenic activity of endothelial cells: direct or VEGF-dependent effect?

Józef Dulak\textsuperscript{a,},\textsuperscript{*} Alicja Józkowicz\textsuperscript{b,c}

\textsuperscript{a}Department of Cell Biochemistry, Institute of Molecular Biology and Biotechnology, Jagiellonian University, Kraków, Poland
\textsuperscript{b}Laboratory of Molecular Genetics and Genetic Engineering, Institute of Molecular Biology and Biotechnology, Jagiellonian University, Kraków, Poland
\textsuperscript{c}Department of Vascular Surgery, University of Vienna, Vienna, Austria

Received 25 June 2002; accepted 25 June 2002

In a recent interesting paper Babaei and Stewart [1] demonstrated that coculture of endothelial cells (ECs) with smooth muscle cells (SMCs) may result in the formation of extensive capillary-like structure. However, the effect was observed only when SMCs were previously transfected with vector containing the endothelial nitric oxide synthase (eNOS) cDNA. Such an in vitro angiogenic-like events were abrogated by L-NAME, a NOS inhibitor. A similar angiogenic response has been observed when SMCs were transfected with plasmids containing VEGF\textsubscript{121} cDNA, the effect again being inhibited by L-NAME. Similarly, in a Boyden chamber model the EC migration was potently enhanced in the presence of SMC transfected with eNOS or VEGF\textsubscript{121}, and was significantly attenuated by L-NAME.

Much evidence indicates that NO plays an integral role in VEGF signaling (see Ref. [1]). It has been convincingly demonstrated that NO may be involved in EC proliferation, migration, protease release and increased vascular permeability, the effects important for initiation of angiogenesis. Thus, the recent report by Babaei and Stewart [1] is in keeping with previous observations. The authors state that this is the demonstration that NO exerts a direct proangiogenic activity. They claim that NO generated by eNOS or by NO donors facilitate EC migration, even in the absence of growth factors [1].

We want, however, to propose another explanation for those phenomena. A number of recent studies demonstrated that NO induces VEGF synthesis in numerous cell types, among them SMCs ([2–6] and Refs. therein). We have shown that induction of inducible NOS (iNOS) [2–4]

\textsuperscript{*}Corresponding author. Tel.: +48-12-252-6375; fax: +42-12-252-6902.

E-mail address: jdulak@mol.uj.edu.pl (J. Dulak).

or treatment of SMCs with different NO donors [5] induces VEGF expression in rat or human VSMCs, acting at the level of transcriptional activation. Additionally, we have recently demonstrated that VSMCs engineered to overexpress plasmids containing either the iNOS [5] or eNOS gene [2,5] generated significantly more VEGF than cells transfected with control vectors. Such an enhanced VEGF synthesis was attenuated when eNOS or iNOS-transfected cells were treated with the NOS inhibitor L-NAME, but not the inactive enantiomer, D-NAME. More importantly, we have finally demonstrated that the conditioned media from SMC transfected with eNOS or iNOS enhanced the proliferation of human umbilical vein endothelial cells (HUVECs), the effect being abrogated by pretreatment with anti-VEGF neutralizing antibodies [5].

In their study, Babaei and Stewart used only inhibitors of NOS activity and did not investigate the possibility that overexpression of eNOS enhanced VEGF production [1]. Thus, we postulate that the direct angiogenic effect of NO has not been yet proven.

The increased morphogenic events observed by Babaei and Stewart in the co-culture of ECs and SMCs transfected with VEGF expression vectors were also abolished by L-NAME. This observations add to the previous studies demonstrating that VEGF induces angiogenesis by enhancing NO generation in endothelial cells [1].

Endothelial cells are the target, not the main source of VEGF. Microvascular endothelial cells express VEGF and can release some amounts of this growth factor. Microvascular endothelial cells, like HUVECs, do not release detectable quantities of VEGF into the culture media. Interestingly, however, it has been also recently demonstrated that exogenous VEGF can induce VEGF expression in microvascular endothelial cells [7]. The detailed mecha-
Fig. 1. Proposed mechanisms of VEGF–NO relationship in activation of angiogenic events in endothelial cells. Increased generation of NO by SMCs, obtained either by genetic augmentation (e.g., eNOS transfection) or by induction of iNOS can enhance VEGF production in VSMCs. VEGF activates its receptor(s) leading to the increased eNOS activity and NO generation in ECs. It remains to be elucidated whether NO exerts a direct pro-angiogenic effect in ECs, or whether its activity is mediated by the autocrine stimulation of VEGF synthesis.

isms behind this event have not been yet clarified. Therefore, it would be worthwhile to elucidate whether the NO-dependent effects in ECs rely on VEGF expression in those cells (Fig. 1). Accordingly, it still remains to be proven whether NO really exerts a direct angiogenic effect in ECs or whether this action of NO is mediated by autocrine activity of VEGF.

Acknowledgements

Studies in the authors’ laboratories are supported by grants from the Polish State Committee for Scientific Research.

References