Single cell analysis and mathematical description of intracellular calcium regulation through VEGF signaling.

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Vascular Endothelial Growth Factor (VEGF) is a highly influential mediator of angiogenesis and has been shown to promote different aspects of endothelial cell behavior including cell proliferation, migration, and differentiation. Although several signaling pathways have been shown to transmit information from VEGF receptors, recent studies have shown that the process of angiogenesis is very sensitive to alterations in intracellular calcium. Here we perform a series of single cell experiments and show that there is a high degree of diversity in the regulation of intracellular calcium following VEGF stimulation. We develop a mathematical model describing the regulation of intracellular calcium via VEGF that is able to reproduce several of the dynamic features associated with transient and prolonged VEGF signaling. In addition, we describe a microfluidic system which allows us to expose cells to VEGF in a time varying manner and illustrate how this approach can be useful in further developing the model.

Keywords **— VEGF, calcium signaling, angiogenesis, FRET**

Angiogenesis, the process of blood vessel sprouting, plays a key role in both normal vascular physiology and disease such as tumor formation and diabetic retinopathy. During these processes, endothelial cells exhibit a variety of different behaviors including differentiation from a quiescent state, proliferation, migration, and tube formation. While numerous factors have been implicated as important to the process of angiogenesis, VEGF has received a great deal of attention due to its ability to promote each of the endothelial cell phenotypes mentioned above.

Although endothelial cells have several VEGF receptors and co-receptors, the second isoform known as KDR is believed to play a pivotal role in transmitting information. Accordingly, numerous studies over the last few decades have characterized the activation of KDR and the signaling cascades it activates. Notably, these pathways include ERK MAPK, p38 MAPK, PI3K, FAK, and Ca^{2+} / nitric oxide (1). Each of these pathways appears to contribute differently to one or more of the phenotypes observed during angiogenesis. More recently, studies have illustrated the importance of calcium signaling in promoting angiogenesis both directly and through modulation of NO production (2,3). Moreover, carboxyamide-triazole (CAI), an inhibitor of non-voltage gated calcium channels, has been shown to prevent neovascularization in both tumor and ischemic retinopathy models (4) and is undergoing clinical trials as an anticancer agent.

In this study, we present both an experimental and mathematical description of calcium regulation by VEGF. Our first aim is to elucidate whether endothelial cells respond to different concentrations of VEGF in a uniform manner or whether attention must be paid to single cell responses. Using FRET analysis, we observed that endothelial cells display a variety of different dynamic responses to the same level of VEGF stimulation through KDR. With this in mind, we then construct a computational model linking VEGF stimulation to calcium regulation. We found that the model is capable of predicting many of the interesting dynamic features we observed and suggest that it will provide a useful tool in further understanding the scope of information transmitted to endothelial cells via VEGF. Finally, we present a microfluidic system that we have recently developed which allows us to expose cells to VEGF in a time varying manner and illustrate how this approach can be useful in further developing the model.

REFERENCES

[1] Claesson-Welsh et al., Biochem Soc Trans (31) 2003
[2] Kohn et al., Proc. Natl. Acad Sci (92) 1995
[3] Bauer et al., Pharm Exp Ther (292) 2000
[4] Franklin et al., Inv Ophth Vis Sc (45) 2004

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