M-CSF-induced VEGF production from human monocytes is regulated by the transcription factor, SP1, and the translation factors, eIF4E and 4E-BP1.

Jennifer M. Curry[1,2] and Tim D. Eubank[1], Ryan D. Roberts[1,2], Yijie Wang[1], Nabendu Pore[3], Amit Maity[3], Clay B. Marsh[1,2]

[1]The Dorothy M. Davis Heart and Lung Research Institute,
[2]Integrated Biomedical Science Program, Department of Internal Medicine, Division of Pulmonary Medicine, and The Ohio State University, Columbus, OH 43210
[3]Department of Radiation Oncology, Philadelphia Veterans Affairs Medical Center, and the University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Recently, we reported that macrophage colony-stimulating factor (M-CSF) induces the expression of vascular endothelial growth factor (VEGF) from human monocytes. This VEGF is biologically active in an *in vitro* angiogenesis assay. In our current study, we show that growth factor-reduced Matrigel resuspended with M-CSF and injected into mice recruits mononuclear phagocytes and endothelial cells to the plug. In addition, we elucidate the signaling pathway responsible for M-CSF-induced VEGF up-regulation. The MEK/ERK inhibitor, U0126, suppresses VEGF production from M-CSF-stimulated monocytes suggesting that the MAP kinase member, ERK1/2, is involved in this signaling scheme. Additionally, immunohistochemical staining for Hypoxia Inducible Factor (HIF) and confocal microscopy suggests that M-CSF-induced VEGF expression is a HIF-independent mechanism as we observed no significant difference in nuclear translocation of HIF-1α or HIF-2α between M-CSF- and non-M-CSF-treated cells. The SP1 inhibitor (mithramycin), DNA binding assays, and VEGF promoter analysis using SP1 sequence mutant constructs indicate that the transcription factor SP1 plays a role in M-CSF–stimulated VEGF production. Lastly, we investigate the eukaryotic initiation factor, eIF4E, and its endogenous inhibitor, 4E-BP1, and found that 4E-BP1 is phosphorylated in response to M-CSF which increases VEGF mRNA translation efficiency. This M-CSF-induced 4E-BP1 phosphorylation is inhibited by U0126, suggesting ERK1/2 involvement in the translational regulation of VEGF production.