The effect of hypoxia on VEGF expression in feline oral squamous cell carcinoma

Madison King, Haili Wang, Luis Garcia, Russell S. Fraser, and Chelsea Martin

Department of Pathology & Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island

Vascular endothelial growth factor (VEGF) is a biomarker for poor prognosis in human oral squamous cell carcinoma (OSCC), but it responds poorly to treatment with anti-angiogenic drugs. VEGF-A and VEGF-D have been found in feline OSCC (FOSCC) tissue samples and cell lines, but VEGF-B and VEGF-C expression have not yet been reported. This study aimed to characterize *VEGF-A*, *VEGF-B*, *VEGF-C*, and *VEGF-D* gene expression in feline FOSCC cell lines following 72 hours of culture in hypoxic conditions (1% O_2 with 5% CO_2) compared to typical cell culture conditions (about 17% O_2 with 5% CO_2). Three FOSCC cell lines (SCCF1: laryngeal, SCCF2: gingival, SCCF3: lingual) and a fibroblast cell line (CRL6167) were selected to represent the FOSCC microenvironment. Renal cortical cells (CCL94) were also included for their mesenchymal characteristics in cell culture. PCR primers were designed and validated in order to evaluate relative *VEGF* mRNA expression semi-quantitatively using reverse transcription PCR. Each FOSCC cell line differed in *VEGF* expression profile and response to hypoxia. *VEGF-B* and *VEGF-D* expression was stimulated by hypoxia in SCCF1 cells, but *VEGF-A* expression was inhibited by hypoxia in SCCF2 cells. There was no statistically significant effect of hypoxia on SCCF3 cells. Conversely, hypoxia stimulated *VEGF-A* and *VEGF-C* expression in fibroblasts. Interestingly, *VEGF-C* expression was much higher in fibroblasts than in any of the FOSCC cells. Future experiments will determine changes in VEGF expression at the protein level, and RNA sequencing will be used to characterize changes in the transcriptome-wide response of FOSCC cells to hypoxia.

Financial support provided by AVC Veterinary Summer Research Award, AVC Internal Research Fund, and an NSERC Discovery Grant.