Hypoxia Effects on Cell Viability and Cytokine Secretion in Vestibular Schwannoma and Human Schwann Cells

Carly Misztal, MD[1], Lucienna Wolf, BS[1], Olena Bracho BS, MBA[1], Bryan Souza, BS[1], Michael Ivan, MD[2,3], Cristina Fernandez-Valle, PhD[4], Christine Dinh MD[1,3]

[1]Department of Otolaryngology, University of Miami, Miller School of Medicine, Miami, FL, USA; [2] Department of Neurological Surgery, University of Miami, Miller School of Medicine, Miami, FL, USA; [3] Sylvester Comprehensive Cancer Center, Miami, FL, USA; [4]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida

Introduction

- **Vestibular Schwannomas (VS)** are benign intracranial tumors arising from Schwann cells (SC) of cochleovestibular nerves. They can cause hearing loss, dizziness, and other intracranial complications. Our lab has previously shown that these tumors can be highly heterogenous in the cytokines they secrete and immune cell expression within the tumor microenvironment. For instance, cystic VS have been shown to have different levels of certain cytokine expression that may be associated with more aggressive clinical features. Current treatment options for VS include observation with serial imaging, radiation, or surgical resection. These treatment options can lead to irreversible neurological sequelae or have limited efficacy, and pharmacotherapy options remain limited.
- **Hypoxia,** or the effect of low oxygen conditions on tumor growth, has been extensively studied in malignant tumors. We know that cancer can outgrow their vasculature and develop hypoxic areas. These tumors undergo metabolic reprogramming and continue to proliferate despite low oxygen conditions. However, the data regarding hypoxia effects on VS remain limited. Studying how VS cell and SC viability and the associated secretome changes in hypoxia may reveal potential targets for pharmacotherapy.

Objective

- Determine the effect of low oxygen tension on cell proliferation and cytokine secretion in SCs and VS cells

Methods

- **Primary VS Cells:** Tumor chunks were harvested prospectively from patients undergoing VS surgery between January 2019 to January 2025. One freshly harvested tumor was randomly selected for this primary study. Tumor was enzymatically dissociated, and primary VS cultures were cultivated in Schwann media (Sciencell).
- **Human Schwann Cells:** Normal human SCs were obtained from lab collaborators and similarly cultivated in Schwann media (Sciencell).
- **Culture Conditions:** Primary VS cells and SCs were placed in a maintenance media consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. Cells were then cultured in normoxic (21% oxygen) and hypoxic (5% oxygen) conditions for 72 hours.
- **Cell Viability:** A viability assay recording luminescence as a measure of ATP production was performed at 72 hours according to manufacturer's protocol (CellTiter-Glo™, Promega). Measurements were recorded as relative luminescent units (RLU)
- **Cytokine Array:** Conditioned media was collected at 72 hours. Secreted cytokines were measured in conditioned media using a 120-cytokine array (Raybiotech), per manufacturer's protocol. Protein expression was measured using chemiluminescence imaging (Jess) and ImageJ software (NIH) with microarray plug-in.

Results

Cell Viability in Hypoxia and Normoxia

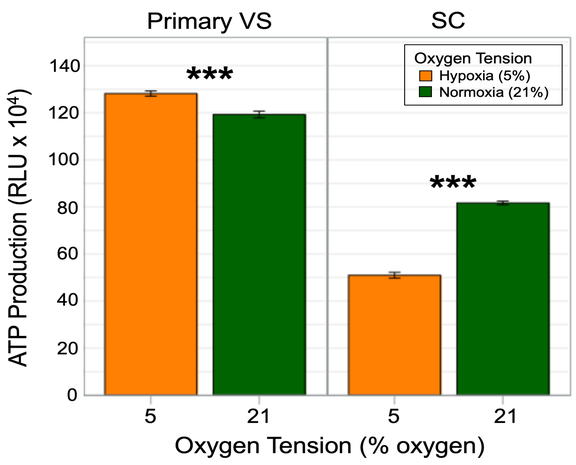

Figure 1. Cell Viability in Hypoxia and Normoxia. Hypoxia caused a significant reduction in viability of normal SCs ($p < 0.0001$) and a significant increase in viability of VS cells when compared to normoxia ($p < 0.001$).

Cytokine Array

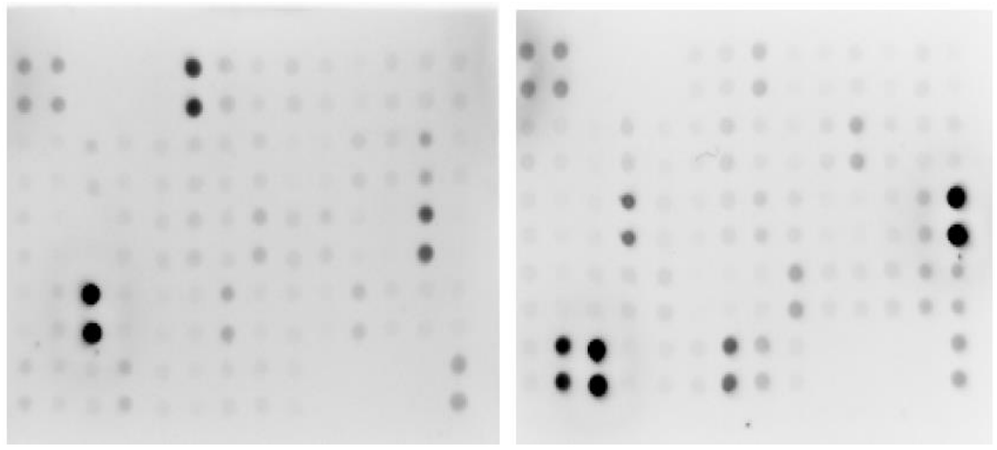

Figure 2. Cytokine Array. A representative image of the two membranes making up the 120-cytokine array, which shows the expression of secreted cytokines from VS in hypoxic conditions.

Conclusions

- VS cells may be more resistant to hypoxia than normal SCs, suggesting that these cells have adapted mechanisms to continue to proliferate in hypoxia.
- Hypoxia induced cell proliferation in VS may be related to the upregulation of IL-8, which is a cytokine that is known to promote cell proliferation and angiogenesis in several cancers.
- We also demonstrated that normal SCs and VS cells upregulate TIMP-1 in hypoxic conditions, which is a protein that has been shown to protect against neuronal death after hypoxic insults. The clinical significance of this finding is unknown.
- VS cells express more MCP-1, highlighting the importance of monocyte-VS crosstalk in the tumor microenvironment in all oxygen conditions. Based on our prior work, this may correlate with different clinical phenotypes.
- Additional studies are warranted to further elucidate how hypoxia affects various VS and related immune cells within the tumor microenvironment, which can lead to the identification of novel therapies for VS.

Secreted Cytokines in Primary VS

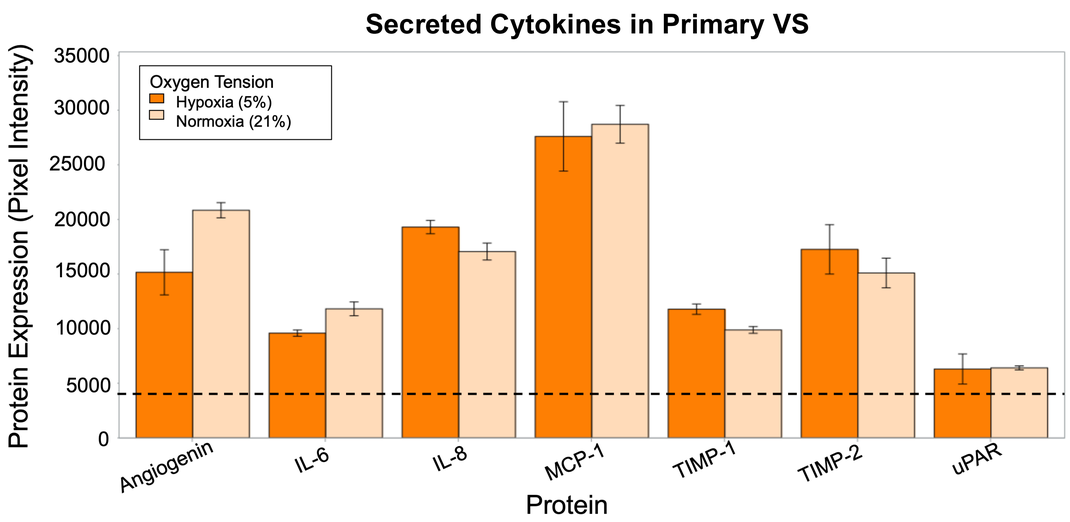

Figure 3. Secreted Cytokines in Primary VS. Seven cytokines were highly secreted from VSB19. Interleukin-8 (IL-8) and tissue inhibitor of metalloproteinases (TIMP-1) were secreted at higher levels in hypoxia, while angiogenin and IL-6 were secreted at higher levels in normoxia. Monocyte chemoattractant protein (MCP-1), TIMP-2, and urokinase plasminogen activator receptor (uPAR) were secreted highly in both conditions.

Secreted Cytokines in Normal SCs

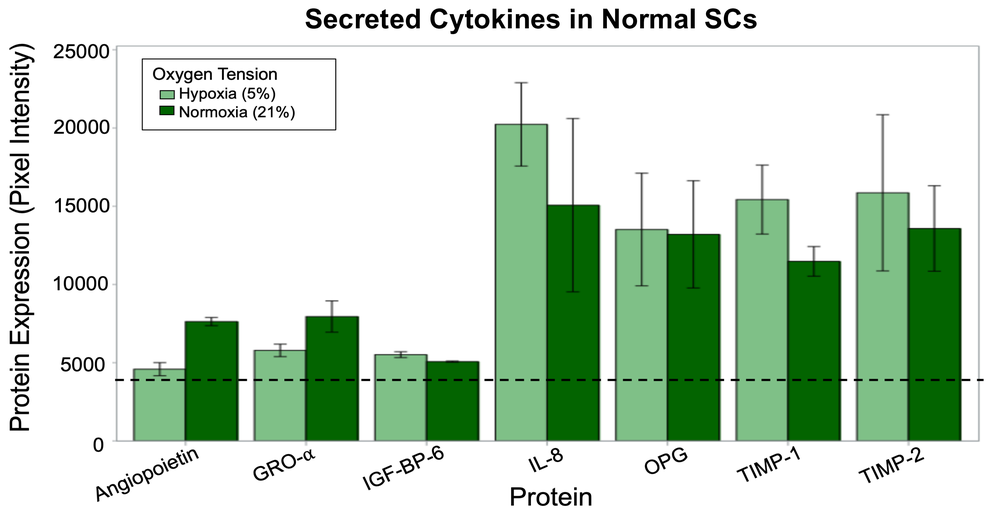

Figure 4. Secreted Cytokines in Normal SCs. Seven cytokines were highly secreted from normal SCs. TIMP-1 and insulin-like growth factor binding protein-6 (IGF-BP-6), was secreted at higher levels in hypoxia, while angiopoietin and growth-related protein-α (GRO-α) were secreted at higher levels in normoxia. Osteoprotegerin (OPG), TIMP-2, and IL-8 were secreted highly in both oxygen conditions.

Contact Information

- Christine Dinh, MD, Professor of Otolaryngology, University of Miami Miller School of Medicine, Address: 1120 NW 14th Street, 5th Floor, Miami, FL 33136, Phone: (305) 243-1484; Fax: (305) 243-2009; Email: ctdinh@med.miamim.edu

Funding

- American Hearing Research Foundation Bernard & Lottie Drazin Memorial grant (C. Misztal)
- Ansin Foundation, Dr. Dinh's lab, University of Miami Department of Otolaryngology

References

- Nisenbaum E, et al. Cytokine Profiling of Cyst Fluid and Tumor-Associated Macrophages in Cystic Vestibular Schwannoma. Otol Neurotol. 2023 Dec 1;44(10):1073-1081.
- Nisenbaum E, Misztal C, Szczupak M, Thielhelm T, Peña S, Mei C, Goncalves S, Bracho O, Ma R, Ivan ME, Morcos J, Telischi F, Liu XZ, Fernandez-Valle C, Dinh CT. Tumor-Associated Macrophages in Vestibular Schwannoma and Relationship to Hearing. OTO Open. 2021 Nov 23;5(4):2473974X211059111. doi: 10.1177/2473974X211059111. PMID: 34870062; PMCID: PMC8638079.
- Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clin Cancer Res. 2008 Nov 1;14(21):6735-41. doi: 10.1158/1078-0432.CCR-07-4843. PMID: 18980965.
- Tejima E, Guo S, Murata Y, Arai K, Lok J, van Leyen K, Rosell A, Wang X, Lo EH. Neuroprotective effects of overexpressing tissue inhibitor of metalloproteinase TIMP-1. J Neurotrauma. 2009 Nov;26(11):1935-41. doi: 10.1089/neu.2009.0959. PMID: 19469687; PMCID: PMC2822804.