The mTOR Inhibitor, CCI-779, Attenuates Angiogenesis In Vitro via the inhibition of mTOR/ STAT3/HIF-1α

Presenting Author	Hillary A. Savage, BFA, Medical Student, MSII, Department of Otolaryngology- Head and Neck Surgery, LSU Health Shreveport, Shreveport, LA
Co-authors	Md. Maksudal Alam, PhD, Post Doctoral Fellow, Otolaryngology HNS, LSU Health Science Center, Shreveport, LA; Janmaris Marin, MD, Clinical Research Associate, Otolaryngology HNS, LSU Health Science Center, Shreveport, LA; Tara Moore-Medlin, BS, Research Specialist, Otolaryngology HNS, LSU Health Science Center, Shreveport, LA; Xiaohua Rong, BS, Research Associate, Otolaryngology HNS, LSU Health Science Center, Shreveport, LA; Cherie-Ann O. Nathan, MD FACS, Professor and Chariman, Otolaryngology HNS, LSU Health Science Center, Shreveport, LA
Disclosure Information	Authors and Co-authors have no relevant financial relationships to declare.
Supplemental Video	Supplemental Video
Category	Medicine & Medical Subspecialties

Abstract

Background: HPV-negative (-) head and neck squamous cell carcinoma (HNSCC) has a recurrence rate of 50-60%, with 75-85% of tumors expressing TP53 mutations.
mTOR inhibitors (mTORi) have been shown to increase progression-free survival in TP53-mutant HNSCC, however, its underlying mechanism remains unclear. One hypothesis is that mutant P53 activates mTOR/STAT3/HIF-1α pathway, which increases the level of VEGF protein thereby inducing neovascularization that causes tumor recurrence. Based on that, the attenuation of these pro-angiogenic cytokines would create a less-than-favorable tumor microenvironment for residual tumor cell survival and proliferation. The goal is to determine the effects of a mTORi on mTOR/STAT3/HIF-1α /VEGF pathway in HPV (-) TP53 mutant HNSCC cell line, to measure the effect of mTORi treatment on HPV (-) TP53 mutant HNSCC mediated in vitro angiogenesis.

Methods: The effect of CCI-779 on STAT3/HIF-1 alpha pathway was analyzed by Western blot. The in vitro effect of CCI-779 on angiogenesis was determined using HMEC cell line. In vitro endothelial cell proliferation was done using MTS assay, while cell migration assay was done using crystal violet staining.

Results: In vitro, CCI-779 significantly ($P<0.05$) decreases the levels of Hif-1α and VEGF-A via the inhibition of mTOR/STAT3/HIF-1α pathway in an HPV (-) TP53 mutant HNSCC cell line when compared with wild type TP53cell line. The ability of CCI-779 to decrease VEGF-A expression also significantly ($P<0.05$) reduces the migration and cell viability of endothelial cells in the conditioned media from an HPV (-) TP53 mutant HNSCC cell line, compared to the wild type TP53 cell line.

Conclusions: In vitro, CCI-779 appears to reduce the expression of HIF-1α and angiogenic cytokine VEGF-A more efficiently in HPV (-) TP53 mutant HNSCC cells, compared to the wild type TP53 cell line which in turn significantly reduces the angiogenic ability of TP53 mutant HNSCC cell line.

Learning Objectives

Describe the need for adjuvant therapy for patients with high risk, HPV (-) HNSCC
Describe the effects a mTOR inhibitor may have on a neovascularization pathway, mTOR/STAT3/HIF-1α, and how this may affect tumor recurrence.