Therapeutic targeting of Hypoxia-inducible factors in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Introduction

Hypoxia-inducible factors (HIFs) are transcription factors which induce gene expression to mediate the physiological response to hypoxia both in normal and disease conditions (Figure 1). Many cancers, including head and neck squamous cell carcinoma (HNSCC), involve the dysregulated activation of HIFs given their involvement in mediating processes such as angiogenesis, glycolysis, cell survival, and cancer stem cell specification.[1,2] This is correlated with increased mortality and resistance to chemotherapy and radiotherapy, making HIFs a suitable target for anticancer therapeutics. We sought to identify novel HIF-1/2 alpha inhibitors and test their efficacy and effects on the tumor immune microenvironment in HNSCC.

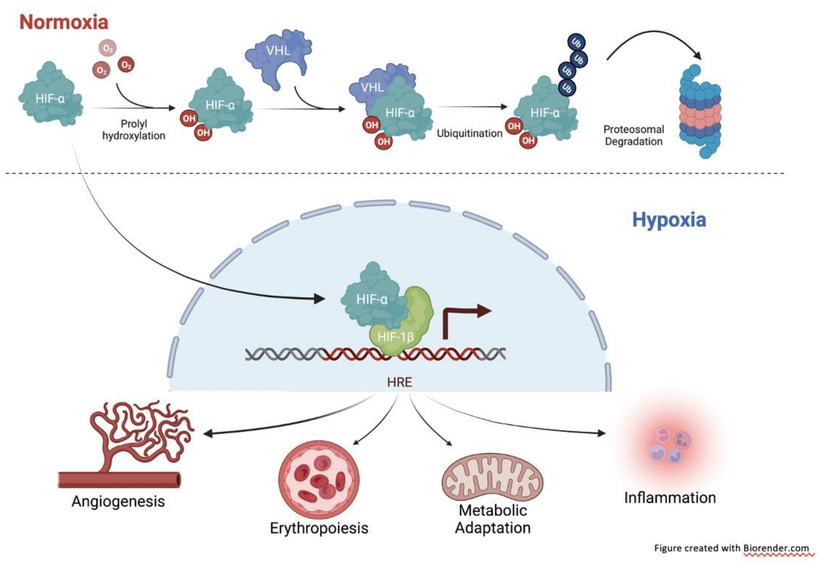

Figure 1. Schematic showing the HIF complex in normoxia and hypoxia, revealing the process of HIF degradation in normoxic environments and in hypoxic environments the process of HIF stabilization and gene transcription.

Methods and Materials

Utilizing virtual and cell-based assays, compounds were identified which disrupted binding of HIF-1α and HIF-2α to HIF-1β leading to suppression of HIF target gene expression. These inhibitors were further validated in the human FaDu and murine SCCVII models using gene expression analyses of HIF target genes. After validation, the inhibitory potential of the most potent SS1.21 was further tested *in vivo* using a syngeneic model. In the syngeneic murine model, the principal endpoint was significant tumor growth inhibition.

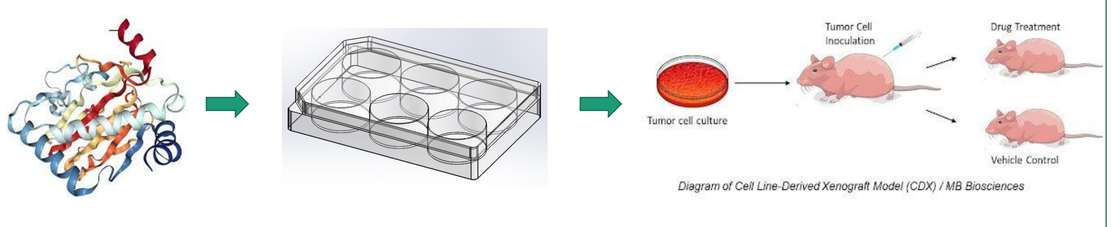

Figure 2. Schematic detailing methods – compounds specifically designed to disrupt HIF complex binding were computationally designed and validated *in vitro* through cell-based assays. Those effective at inhibiting HIF target genes were then tested *in vivo* in both a xenograft and syngeneic mouse model.

Results

Two compounds, identified as 1.21 and 3.2, were discovered through a cell-based assay and subsequently advanced to gene expression and *in vivo* studies in the HNSCC model. The gene expression studies revealed that adrenomedullin (Adm), a well-known hypoxia-inducible gene, was significantly upregulated under hypoxic conditions. Compound 1.21 effectively inhibited the expression of Adm in a dose-dependent manner, with an IC_{50} of 0.03 μM. In comparison, compound 3.2 inhibited Adm expression with an IC_{50} of 1.1 μM.

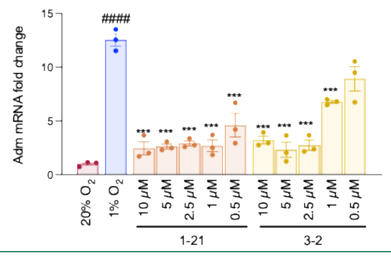

Chart 1. Gene expression data for compounds, 1.21 and 3.2, in SCCVII *in vitro* assays demonstrating significant inhibition of the hypoxia inducible gene, adrenomedullin (Adm).

Results

The compound 1.21 was then studied *in vivo* using syngeneic murine models, and xenograft models which revealed a significant reduction in tumor growth at concentrations of 10 mg/kg and 20 mg/kg in both the human FaDU and mouse SCCVII models.

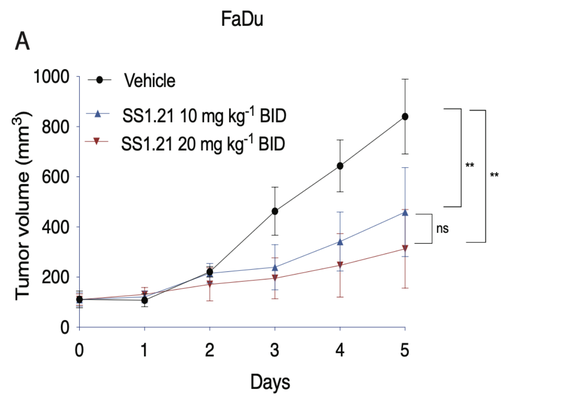

Figure 3. Graph depicting tumor growth over 5 days of treatment in the xenograft model. Treatment with 20 mg/kg twice daily showed approximately 75% inhibition of tumor growth.

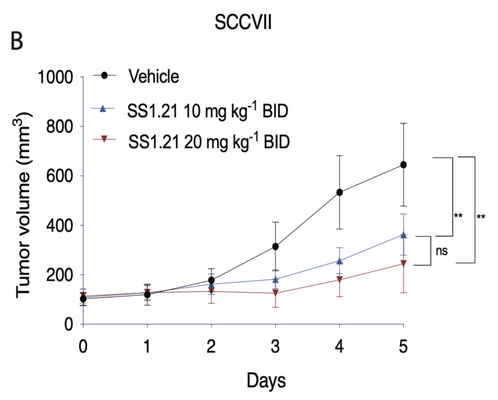

Figure 4. Graph depicting tumor growth over 5 days of treatment in the syngeneic model. Treatment with 20 mg/kg twice daily showed approximately 75% inhibition of tumor growth.

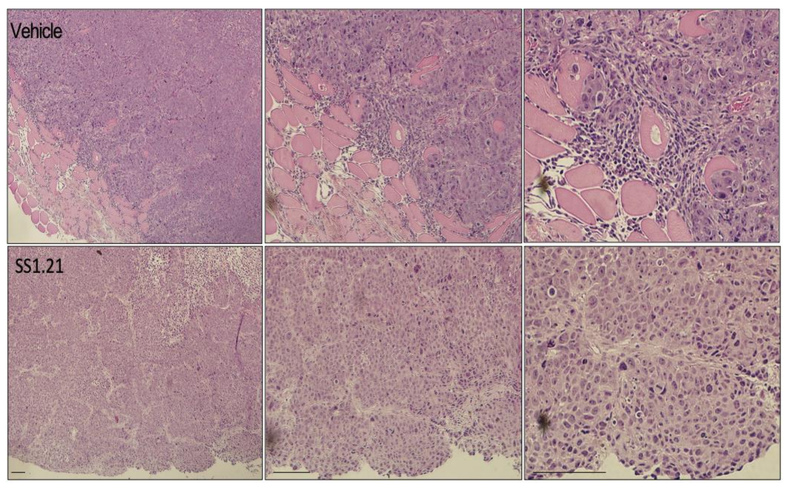

Figure 5. Histology from tissue harvested from animals with FaDu tumors treated with the HIF inhibitor vs. vehicle showed a decrease in tissue infiltration compared to vehicle treated mice.

In the FaDu model, tissue harvested from vehicle treated and those mice treated with HIF inhibitor 1.21 showed noticeable histological differences – with the HIF inhibitor treated tumors well demarcated with clean resection margins as opposed to the vehicle treated tumors which were difficult to resect due to extensive infiltration of surrounding skeletal muscle by cancer cells

Discussion

Head and Neck Squamous Cell Carcinoma is the 6th most prevalent malignant cancer worldwide with rates of mortality of 40-50%.[3] Current treatment regimen primarily involves the use of radiotherapy often in conjunction with chemotherapeutic drugs such as cisplatin. HIFs have been noted to affect cancer progression while also inhibiting treatment by causing tumors to be more radioresistant.[4] Developing a targeted treatment towards HIFs has the promise to stifle metastatic spread of HNSCC while also contributing to improved response to current standard of care treatment modalities such as radiotherapy.

Conclusions

Treatment with HIF-inhibitors can alleviate tumor burden and have a potent effect on HNSCC tumor growth inhibition as shown in this murine model, warranting further studies involving combination treatment studies utilizing immunotherapy and radiation therapy.

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