PX-12 Synergistically Enhances the Therapeutic Efficacy of Vorinostat Under Hypoxic Tumor Microenvironment in Oral Squamous Cell Carcinoma *in Vitro*

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ABSTRACT

Introduction: Squamous cell carcinoma of the oral cavity (OSCC), a solid tumor, is known to be resistant to therapy and has an adverse clinical outcome because of its hypoxic environment. The hypoxic tumor microenvironment is majorly regulated by hypoxia inducible factor 1α (HIF-1α), which has been identified as a primary therapeutic target (Ramachandran *et al*., 2015). Histone deacetylase inhibitors such as Vorinostat destabilize HIF-1α through epigenetic regulation. On the contrary, PX-12, a thioredoxin-1 (Trx-1) inhibitor, indirectly destabilizes HIF-1α by downregulating Trx-1. HDAC is are promising agents for cancer therapy, although the potential of HDAC is as monotherapies is limited in solid tumors. Combination of HDACis with other agents has been demonstrated to be useful in potentiating anticancer effects (Wang *et al*., 2021) Vorinostat triggers apoptosis and slows tumor cell proliferation but also raises levels of ROS, a mechanism that PX-12 further potentiates through the Trx-1 pathway. A study showed that the specificinhibition of Trx-1 induces ROS-dependent apoptosis in OSCC celllines, HSC6 and CAL33. Trx-1 inhibition, mediatedbythe ROS pathway, in oral potentiallymalignantdisorders (OPMDs), can delay hypoxia-inducedtransformation to oral malignancy (Chen *et al*., 2018). So, PX-12 (a specific Trx-1 inhibitor) could be used as a potential therapeutic agent to induce apoptosis by ROS pathway in OSCC cells under hypoxic conditions This study suggests that the combination of vorinostat and PX-12 would enhance therapeutic efficacy in OSCC.

Objective: This study is designed to determine the *in vitro* therapeutic potential of combining vorinostat and PX-12 in OSCC cells under conditions of normoxia as well as hypoxia.

Methodology: Cells were plated on 96-well plates and made hypoxic by adding 200 µM cobalt chloride (CoCl2) to the media. The EC50 levels of both drugs were evaluated through serial dilution titration of samples containing the cells with vorinostat as well as PX-12 individually. Drug mix matrices for combination therapy were plated at four-fold, two-fold, one-fold, and 0.25 fold concentrations. The viability of cells was quantified by the MTT assay, and absorbance at 570 nm. Drug combinations were determined using the Chou-Talalay model interaction to obtain the combination index: CI < 0.8 reveals synergism, CI 0.8–1.2 is additive, and CI > 1.2 is antagonistic. Isobologram analysis supported these drug interaction profiles. All experiments were done in triplicate and subsequently analyzed for statistical significance in EC50 values using GraphPad Prism V.

Result: The EC50 values of the vorinostat and PX-12 were found under normoxic conditions as 2.1 µM and 1.8 µM, respectively. When conditions changed to hypoxic, values dropped drastically to 1.2 µM for vorinostat and to 1.0 µM for PX-12. Under hypoxic conditions, when used in combination, the EC50 values both were dropped to 0.3 µM for vorinostat and to 0.4 µM for PX-12, indicating greater efficacy. The combination showed additive effect (CI=0.92) under normoxia and synergism under hypoxia (CI = 0.55). This means the interaction under hypoxia has been potentiated rather than the effect seen under normoxia. Therefore, increased levels of ROS production and disruption of Trx-1 activity would likely be responsible for cancer cells death.

Conclusion: This study demonstrates that combining the therapeutic agents vorinostat and PX-12 exhibits synergism under hypoxic conditions, potentially yielding a therapeutic approach against OSCC. The balance of activity of pathways involving HIF-1α destabilization and ROS regulation by this combination underlies the gain in therapeutic strategy overcoming resistance in solid tumors. Results presented above necessitate further probing of the molecular mechanism of interaction and the possibility of this combination as a clinical application in OSCC and other cancers.

Keywords: Combination Therapy, Therapeutic Resistance, Vorinostat, Hypoxia, PX-12.

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