A potential role for pioglitazone in the chemosensitization of human sinonasal undifferentiated cancer cells (SNUC)

Wade Swenson, Beverly Wuertz, Wendy Miller and Frank G. Ondrey

Molecular Oncology Program, Department of Otolaryngology, University of Minnesota, Minneapolis, MN

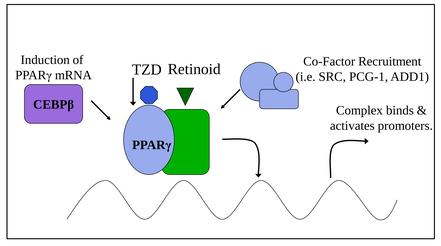
INTRODUCTION

Sinonasal undifferentiated cancer (SNUC) is a rare and aggressive malignancy of the paranasal sinuses. Tumors are frequently advanced at presentation and outcomes have historically been poor with a median survival time of less than 18 months. Multimodality treatment commonly includes surgical resection (when possible), radiation and chemotherapy. The most commonly used chemotherapy agents used are cisplatin and 5-fluorouracil. Despite such aggressive treatment regimens, prognosis remains dismal.

Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the nuclear hormone superfamily and has multiple endogenous and pharmacological ligands, such as pioglitazone. PPARγ agonists regulate development, cellular growth and metabolism in various tissues and pioglitazone has been shown to decrease cellular proliferation, induce differentiation and apoptosis in various tumor phenotypes. In some cases, PPARγ agonism has resulted in sensitization to chemotherapeutic agents.

No prior study has evaluated PPARγ in SNUC. We hypothesized that *in vitro* treatment of a SNUC cell line with pioglitazone would result in decreased cellular proliferation and increased apoptosis, and that these effects would be synergistic with co-treatment of cells with cisplatin.

Fig. 1: Activation of PPARγ and differentiation pathway



MATERIALS & METHODS

Cell Culture
A stable human-derived SNUC cell line, MDA8788-6, gifted by Ehab Hanna, M.D. , (MD Anderson Cancer Center, Houston, TX) was utilized. Cells were grown on tissue culture flasks in DMEM supplemented with 10% head-inactivated FBS and incubated at standard conditions.

Reporter Gene Assay
Cells were plated at $5x10^5$ cells/well in 12-well plates and transiently co-transfected via TransIT LT1 with PPARγ Reporter Gene, thymidine kinase luciferase containing reporter plasmid with a PPARγ response element (PPRE), PPREx3-TK-Luc, a kind gift from Dr. Ronald Evans (The Salk Institute, San Diego, CA and βgal plasmids overnight. After 24hr treatment, cell lysates were analyzed via Tropix Dual Light Reporter Gene Assay on a Tristar dual injection flash luminometer.

MTT Assay
Cells were plated at $5x10^3$ cells/well in 96 well plates and treated the following day and a plate was assayed for input value. MTT was added 24, 48, and 72 hrs after treatment and allowed to incubate at 37^0 for 4 hrs. Crystals were solubilized and the absorbance read at 560nm.

Caspase 3/7 Assay
Cells were plated at $5x10^3$ cells/well in black, clear bottom, 96 well plates and treated the following day. After 24 h incubation, plate was assayed via Caspase 3/7-Glo kit according to manufacturer's instructions (Promega).

RESULTS

Fig. 2: PPAR response element reporter gene assay. A dose-dependent increase in PPARgamma ligand binding activity was seen with Pioglitazone treatment of the SNUC cells (* = P<0.0001)

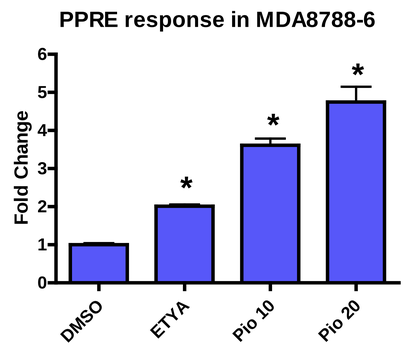


Fig. 3 MTT Assay. Cellular proliferation was reduced with Cisplatin (2-20 microM) treatment and an even further reduction in cellular proliferation was seen with Cisplatin and 10microM pioglitazone co-treatment (P <0.001) at 48 and 72 hours.

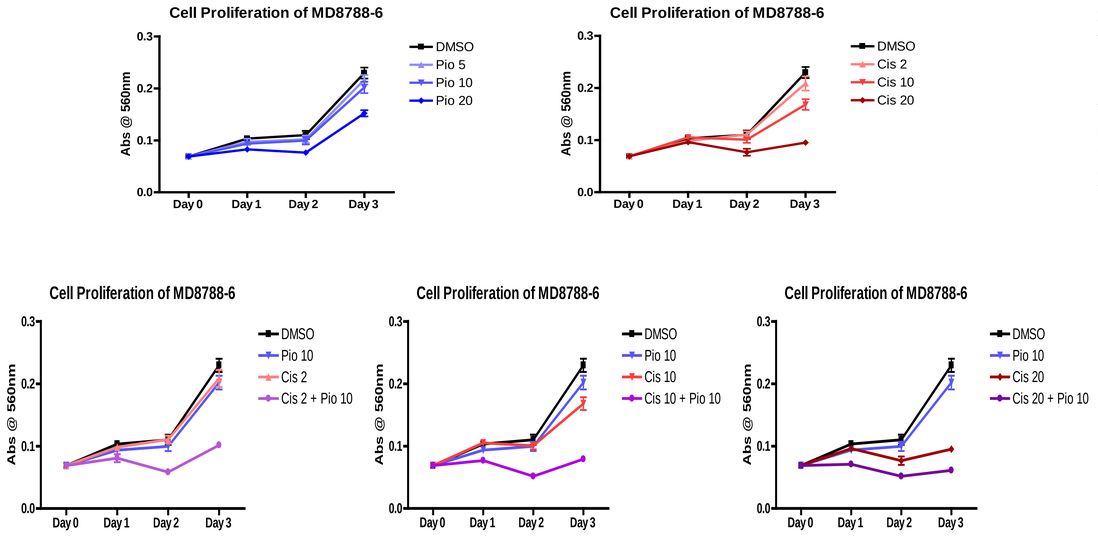
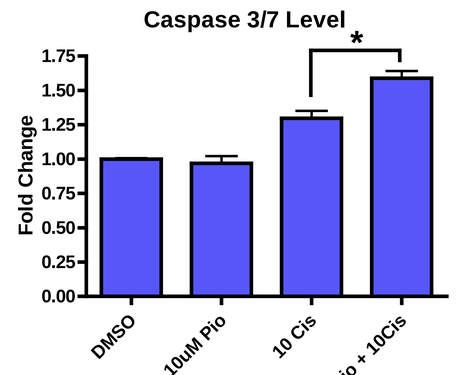


Fig. 4: Caspase 3/7 assay of celluar apoptosis. Pioglitazone co-treatment with 10microM Cisplatin significantly increased cellular apoptosis (* = P = 0.0180) when compared to Cisplatin alone. [DMSO vs Pio P = 0.5921 (ns), DMSO vs 10uM Cis P = 0.0058, DMSo vs combo P = 0.0004]



CONCLUSIONS

We determined that pioglitazone treatment of a SNUC cell line increased PPARγ ligand binding, decreased cellular proliferation increased cellular apoptosis. Co-treatment of the tumor cells with pioglitazone and cisplatin further enhanced the anti-proliferative, pro-apoptotic activity compared to cisplatin-alone. These results are encouraging and support a potential role for PPARγ agonism in the treatment of SNUC.

Further study is needed to determine whether this finding could ultimately translate to chemo-sensitization and improved prognosis in patients receiving chemotherapy for SNUC. Future research efforts will aim to replicate findings in animal models, as well as further elucidate and characterize pertinent molecular pathways involved in PPARγ agonism and SNUC.

ACKNOWLEDGEMENTS

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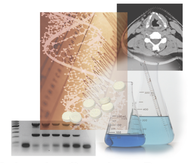

Molecular Oncology Program
Department of Otolaryngology
University of Minnesota