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

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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 pharmacological ligands, such as pioglitazone. PPARγ agonists regulate the nuclear hormone superfamily and has multiple endogenous and agonism and SNUC.

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.

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% heat-inactivated FBS and incubated at standard conditions.

Reporter Gene Assay

Cells were plated at 5x10^4 cells/well in 12-well plates and transiently co-transfected via TransIT LT1 (Wade Swenson, Beverly Wuertz, Wendy Miller and Frank G. Ondrey) with PPARγ response element (PPRE), PPREx3-TK-Luc, a kind gift from Dr. Ronald Evans (The Salk Institute, San Diego, CA) and 8µl pSV-β-gal plasmids overnight. After 24hr transfection, cell lysates were analyzed via Tropix Dual Light Reporter Gene Assay on a Titrion dual detection flash luminometer.

MTT Assay

Cells were plated at 5x10^4 cells/well in 96 well plates and treated the following day for 48 or 72hrs. Cells were washed with PBS and then treated with Cisplatin 2µM and/or pioglitazone 10µM for the desired number of hours. After 48 hrs, cells were fixed with 10% formalin for 30min. Unfixed cells were stained with crystal violet and absorbance read at 560nm.

Caspase 3/7 Assay

Cells were plated at 5x10^4 cells/well in black, clear bottom, 96 well plates and cultured the following day. After 24 hrs 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) when compared to Cisplatin alone. [DMSO vs Pi P = 0.5921 (ns), DMSO vs 10µM Cis P = 0.0008, DMSO vs combo P = 0.0004]

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 10µM pioglitazone co-treatment (P <0.001) at 48 and 72 hours.

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.

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