Effect of Triptolide on MDM2 Expression in HPV-Positive Squamous Cell Carcinoma

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INTRODUCTION

Patients with human papilloma virus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) respond better to standard chemo-radiation therapy than those with HPV-negative status. Mutation of p53 is common found in HNSCC. Triptolide is a diterpene triepoxide derivative from the traditional Chinese herbal plant *Tripterygium wilfordii* hook used to treat autoimmune disease. Previous work demonstrate triptolide and Minnelide (its water soluble pro-drug) reactivate wild-type p53 tumor suppressor gene in HPV-positive HNSCC *in vitro* and *in vivo*, inducing cell death. The mechanism of how TPL reactivates p53 is, however, unknown. The murine double minute 2 (MDM2) protein is a known regulator of p53 activity. MDM2 positivity and p16 seronegativity in esophageal squamous cell carinoma (SCC) is associated with decreased chemosensitivity. Others have shown an inverse relationship between the presence of p16, a surrogate marker for HPV, and MDM2 in laryngeal SCC.

The present study: 1) examines the association between HPV status and MDM2 expression HNSCC specimens and 2) MDM2 activity in cell lines following treatment with TPL and cisplatin.

MATERIALS & METHODS

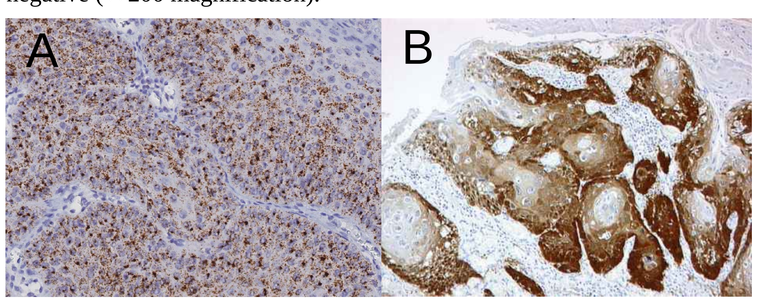
HPV-positive SCC cell line UMSCC 47 and HPV-negative SCC tumor cell line UMSCC 11A were cultured, then incubated with dimethyl sulfoxide (solvent vehicle), cisplatin concentrations (2.5 µM), triptolide (100 nM), or cisplatin plus triptolide combination treatments for 24-72 hrs. MTT was added 24, 48, and 72 hrs after treatment and allowed to incubate at 37°C for 4hrs.

18 HPV positive and 18 HPV negative HNSCC tumors from parafin blocks were sectioned. Unstained sections were de-paraffinized and rehydrated using standard methods. Subsequent steps were automated using an immunohistochemical staining platform (Nemesis, Biocare). Slides were rinsed then blocked (Background Sniper, Biocare Medical, Concord, CA). Mouse monoclonal anti-MDM2 (Sigma, 1:1000) was applied and incubated (Fig. 1).

Whole cell lysates (prepared using the standard RIPA buffer technique) per lane was separated on a 4–12% BisTris NuPage Gel and transferred to PVDF membrane. After blocking for one hour, membranes were blotted with 1° antibody to MDM2 overnight. 2° antibody with fluorophore was incubated for 1 hour then scanned via Li-Cor Odyssey system.

Data analyses and t-tests were performed via SAS version 9.3 and Graph Pad Prism.

Fig. 1: MDM2 immunohistrochemical staining of HNSCC. Tumor cells positive for MDM2 (A) HPV-positive (x400 magnification) (B) HPV-negative (×200 magnification).



RESULTS

Table 1: Expression of MDM2 in HPV-positive and -negative tumors. The mean scores were not statistically significant at the usual p-value cutoff of <0.05, but there was a trend for negative HPV to have higher scores than positive HPV (p<0.10). This is based on the two-sample t-test assuming equal variances. The scores for both negative and positive HPV status did not significantly deviate from a normal distribution. A non-parametric Wilcoxon rank sum test derived from score rankings also resulted in a p-value of 0.082.

HPV status	N	Mean (SD)	Median [min,max]	P-value*
Negative	18	155.3 (70.6)	165 [40,280]	0.082
Positive	17	111.2 (74.7)	120 [10, 260]	

Table 2: Expression of MDM2 in HPV-positive and -negative oropharyngeal tumors subset. The cases were divided up into two groups based on origin. Those in a group labeled "OP" contained the majority of the cases (N=26) and those in the "other" group included the supraglottis, larynx, posterior pharynx, glossoepiglottic fold and buccal (n=7). There were two cases missing an origin. All of the cases in the "other" group were negative for HPV. The following table compares the oropharyngeal tumor subset group between positive and negative HPV status:

HPV status	N	Mean (SD)	Median [min,max]	P-value*
Negative	10	140.5 (63.2)	145 [40,240]	0.315
Positive	16	110.6 (77.2)	105 [10, 260]	

Fig. 2: Combination therapy decreases cell proliferation as effectively as higher dose cisplatin. Triptolide in combination with low dose cisplatin demonstrated greater or equal decreases in cell proliferation as the higher dose cisplatin alone. HPV positive cells demonstrated a more robust response to the combined therapy.

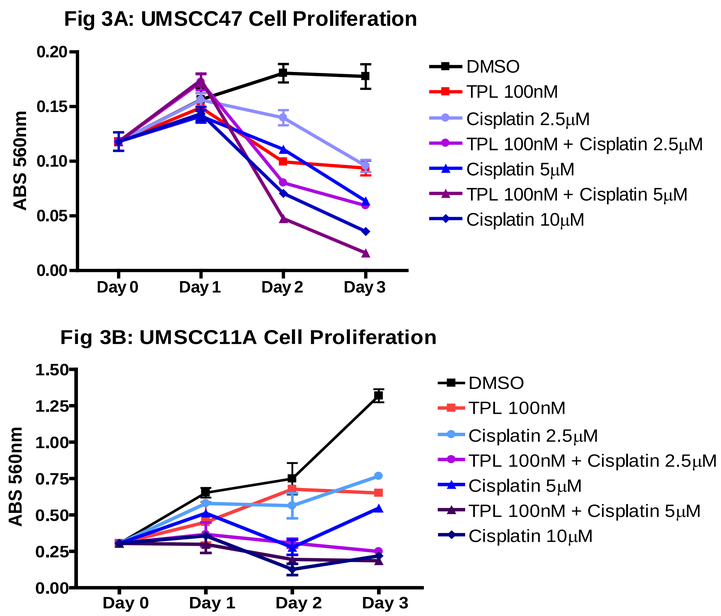
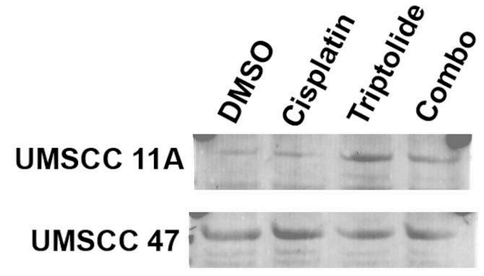


Fig. 2: MDM2 expression following treatment with triptolide, cisplatin, and combination treatments. UMSCC 11A and 47 cells were treated with DMSO (vehicle control), 2.5µM Cisplatin, 100nM triptolide, or the combination. Triptolide trended toward a decrease of MDM2 in UMSCC 47 cells and an increase in MDM2 in UMSCC 11A cells.



CONCLUSIONS

HPV-associated HNSCC has emerged as a sub-set of patients who respond better to concurrent chemo-radiation therapy. However, the toxicity experienced by patients continues to be substantial. We demonstrated TPL, a novel chemotherapeutic agent, can act as a chemo-sensitizing agent *in vitro*, allowing delivery of low dose cisplatin. This chemotherapeutic effect is most robust in HPV-positive HNSCC cell lines. TPL demonstrates less toxicity to non-tumor cells, *in vitro*, suggesting it can represent a useful agent in treatment of HNSCC. The mechanism proposed is reactivation of wild-type p53 via inhibiting the action of MDM2.

The current study supports previous findings with a trend towards increased MDM2 expression in HPV-negative HNSCC tumors. Our data suggests that TPL may reactivate wild type p53 in a MDM2 dependent pathway in HPV-positive HNSCC. TPL decreases proliferation of HPV-negative tumor cells in an p53 independent pathway.

Future studies are needed to investigate MDM2 expression in a larger number of tumors as our data showed a trend but not significantly different due to the small sample size. Additional experiments directed to clearly define the specific MDM2 mutation or polymorphism that attenuates the p53 pathway in HPV-negative HNSCC tumors as well as the efficacy of TPL compared to specific MDM2 inhibitors such as Nutlin-3 would provide additional insight.

ACKNOWLEDGEMENTS

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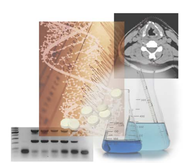

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