Dual inhibition of BER by TRC102 and PARP inhibitor (ABT 888) synergistically enhances cytotoxicity of TMZ in human melanoma

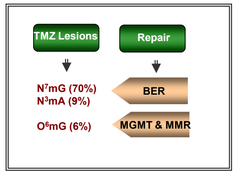
Alina D. Bulgar, Yanling Miao, Stanton L. Gerson, Lili Liu
Case Western Reserve University, Case Comprehensive Cancer Center, Cleveland, OH

Abstract

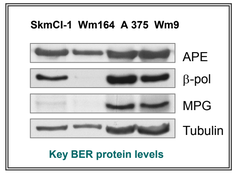
Melanoma, the most fatal skin cancer, has increased in incidence by 15-fold in the past 40 years, the fastest rate increase of any human malignancy. This disease metastasizes rapidly and is highly resistant to chemotherapy and other treatments. Currently, temozolomide (TMZ) is an important part of treatment regimens for advanced metastatic melanoma. However, drug resistance often results in treatment failure. A major resistance factor is the presence of elaborate mechanisms of DNA repair. We have previously studied the role of base excision repair (BER) in conferring TMZ resistance and explored a combined therapy by targeting BER with methoxyamine (TRC102), an inhibitor of BER, which binds to abasic sites and disrupts the BER pathway. The potentiation of TMZ by TRC102 has been validated in different tumor types *in vitro* and in xenograft models. Results showed TRC102 potentiation of TMZ was accompanied by a remarkable induction of DNA strand breaks. In this study, we examined the therapeutic efficacy of the combination of TMZ with TRC102 and a PARP inhibitor (ABT888) in three melanoma cell lines (A375, WM9, and WM164) and in melanoma xenografts. PARP, an enzyme critical in BER pathway, is actively involved in the repair of DNA single and double strand breaks. We hypothesized the inhibition of PARP would inhibit BER with the accumulation of large numbers of unrepaired DNA strand breaks and the combination of TRC102 and ABT888 would synergistically enhance the anti-tumor effect of TMZ through dual inhibition of BER. Results showed that compared to TMZ alone, the combination with either ABT888 or TRC102 more effectively inhibited cell viability and induced apoptosis in A375 and WM9, but not in WM164 melanoma cell lines. The resistance in WM164 cells is probably related to a deficiency in methylpurine-DNA glycosylase, which is responsible for removing TMZ-induced methylated DNA adducts (N^7mG and N^3mA) and producing toxic AP sites. When combined with TMZ and TRC102, ABT888 at a concentration of 5 μM efficiently potentiated cytotoxicity by 8-10 fold in A375 and WM9 melanoma cell lines, respectively, and 4 fold in WM164 cells. Cytotoxicity was correlated with the induction of DNA single and double stranded breaks as assayed by comet assay and induction of γ-H2AX. For *in vivo* studies, the therapeutic regimen was initiated when tumor xenografts (WM9) in nude mice reached ~100 mm³ in volume and treatment was continued for 5 days. Tumor volume was measured for assessment of therapeutic effect. At termination, we found 30-40% reduction in tumor volume following treatment with the combination of TMZ with ABT888 or with TRC102, and 80% reduction in tumor volume by combining the three drugs relative to the TMZ alone group, which had no significant differences in tumor growth in comparison to untreated group. Data indicate that combining a PARP inhibitor with TMZ and TRC102 results in greater inhibition of BER and induces a synergistic cytotoxic effect.

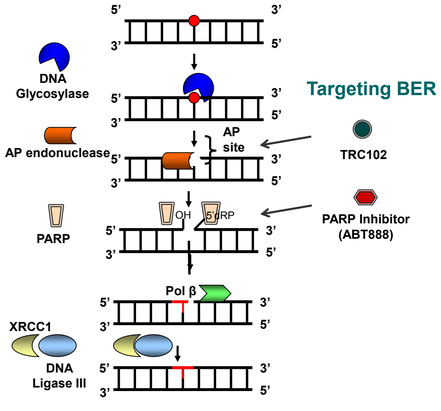
Background

Base excision repair pathway in processing of TMZ damage



Differential expression of BER systems in melanoma cell lines





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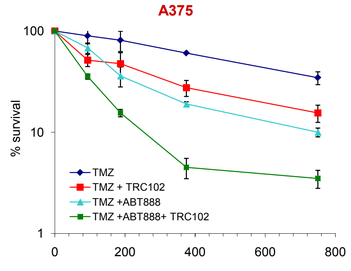
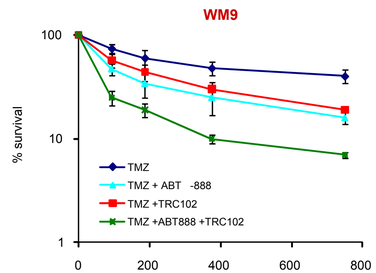
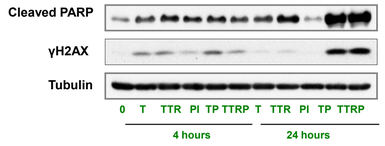
Results

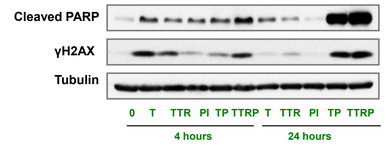
***Hypothesis** : The inhibition of PARP would block BER with the accumulation of large numbers of unrepaired DNA strand breaks and the combination of TRC102 and ABT888 would synergistically enhance the anti-tumor effect of TMZ through dual inhibition of BER.*

PARP inhibitor (ABT888) enhances TMZ and TRC102 cytotoxicity in melanoma cell lines

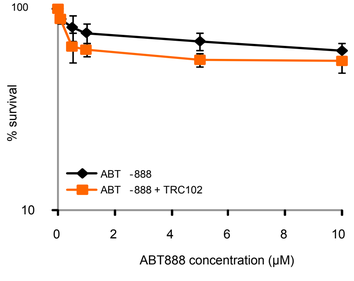


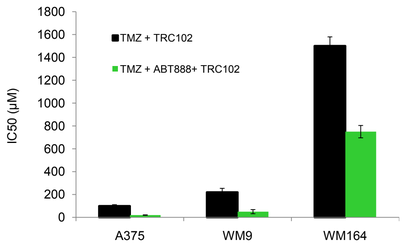




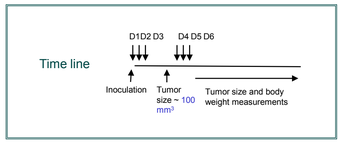


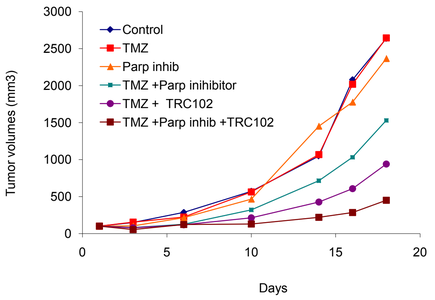
T= TMZ; TR=TRC102; P=PARP inhibitor



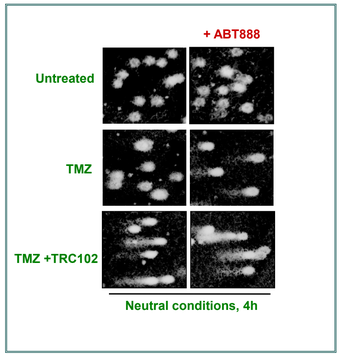


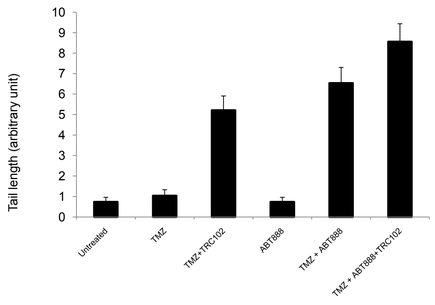
PARP inhibitor (ABT-888) potentiation of TMZ cytotoxicity in human melanoma xenografts

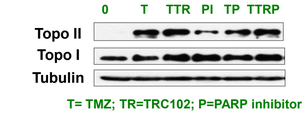




Enhanced induction of DNA strand breaks when PARP inhibitor (ABT888) is combined with TMZ and TRC102

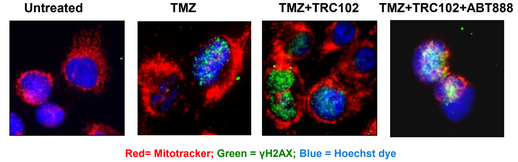






T= TMZ; TR=TRC102; P=PARP inhibitor

γH2AX foci formation after combined treatments



Red= Mitotracker; Green = γH2AX; Blue = Hoechst dye

Summary

- PARP inhibitor (ABT-888) enhances cytotoxicity of the combination of TRC102 and TMZ in human melanoma cell lines and xenografts due to accumulation of unrepaired DNA strand breaks
- PARP inhibitor (ABT-888) has no toxicity alone or in combination with TRC102