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The poly(ADP-Ribose) polymerase inhibitor ABT-888 reduces radiation-induced nuclear EGFR and augments head and neck tumor response to radiotherapy ☆

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ABSTRACT

Background and purpose: Current therapies for head and neck cancer frequently are not curative, necessitating novel therapeutic strategies. Thus, we studied whether inhibition of poly(ADP-Ribose) polymerase (PARP), a key DNA repair enzyme, could improve efficacy of radiotherapy in human head and neck cancer.

Materials and methods: UM-SCC1, UM-SCC5, UM-SCC6, and FaDu human head and neck cancer cellular susceptibility to the PARP inhibitor (PARPi) ABT-888 and/or radiation (IR) was assessed using colony formation assays. DNA damage was evaluated using the alkaline comet assay and immunostaining for γ-H2AX foci. Non-homologous end-joining (NHEJ) mediated repair was measured using phospho-DNA-Pk foci. Epidermal growth factor receptor (EGFR) location was assessed by immunostaining. Poly ADP-Ribose polymerization (PAR) levels were assessed using immunoblotting.

Results: Human head and neck cancer cells exhibited enhanced cytotoxicity with IR and ABT-888 compared to either agent alone. This increased susceptibility correlated with reduced nuclear EGFR, attenuation of NHEJ, and persistence of DNA damage following IR. Interestingly, a subset of head and neck cancer cells which had elevated basal PAR levels was susceptible to PARPi alone.

Conclusions: Combining radiotherapy and PARP inhibition may improve outcomes and quality of life for head and neck cancer patients treated with radiotherapy. Furthermore, this novel strategy may also be feasible in other tumor types. Moreover, PAR levels should be investigated as a potential biomarker for tumor susceptibility to PARP inhibition.

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Agents which target cancers that are deficient in homologous recombination (HR)-mediated DNA double strand break (DSB) repair, such as poly(ADP-Ribose) polymerase (PARP) inhibitors (PARPi), have gained recent attention due to their highly selective killing of BRCA-associated, DNA repair defective tumors while maintaining minimal toxicity in normal tissues [1–3]. Additionally, PARPi has been reported to enhance cytotoxicity in sporadic tumors when combined with other DNA damaging agents, such as with platinum and cyclophosphamide in breast cancer and with temozolomide in glioblastoma [4]. Thus, much effort has been undertaken to expand the utility of PARPi beyond the realm of BRCA-associated tumors by combining with agents that alter the DNA damage/repair pathways.

Even when head and neck cancers have not metastasized, locoregional therapy is frequently not successful. This may, in part, be due to overexpression of the epidermal growth factor receptor (EGFR), which has been implicated in tumorigenesis and disease progression through modulation of proliferation, differentiation, and DNA damage response. Treatment of head and neck cancer typically involves a combination of surgery, chemotherapy, and radiation therapy. It has been reported that, in response to radiotherapy, EGFR is transported to the nucleus and plays a role in radioresistance, poor prognosis, and treatment failures [5–7]. Although targeted chemotherapy and radiation fractionation have had favorable impact, outcomes still remain poor, necessitating novel treatment strategies [8–12].

Thus, in this current study, we hypothesized that enhanced cytotoxicity of head and neck cancer may be achieved by combining radiation (IR), an integral part of therapy that induces cellular DNA damage, and inhibition of PARP, which would theoretically inhibit repair of DNA damage and result in enhanced tumor cytotoxicity.

Consistent with our hypothesis, significantly enhanced cytotoxicity was observed with combination IR and the PARPi ABT-888. This correlated with attenuation of DNA-Pk dependent NHEJ and subsequent persistence of DNA damage. Further dissection of the

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mechanism of inhibited NHEJ revealed that PARPi attenuated nuclear EGFR levels following IR. Interestingly, a subset of head and neck cancer cells were susceptible to PARPi alone and correlated with an elevated basal poly ADP-Ribose polymerization (PAR) level.

These data support the use of the PARPi ABT-888 in combination with radiotherapy as an innovative treatment strategy to potentially improve outcomes in head and neck cancer patients. Moreover, elevated PAR levels may be used to profile and stratify head and neck cancer patients who may subsequently demonstrate superior response to PARP inhibition. Similarly, γ-H2AX levels may be quantified in patient tumor samples to evaluate patient's response to therapy. Furthermore, this strategy may also be feasible in other tumors such as breast, brain, and lung.

Materials and methods

Cell culture

The human head and neck squamous carcinoma cell lines (HNSCC) UM-SCC1, UM-SCC5, and UM-SCC were obtained courtesy of Dr. Thomas E. Carey (University of Michigan, Ann Arbor, MI). FaDu (HTB-43) was obtained from ATCC (Manassas, VA). The PARP inhibitor ABT-888 (Enzo Life Sciences) was utilized in our study. Please refer to SI materials and methods for cell culture growth conditions.

Immunofluorescence

Head and neck cell lines were cultured and seeded on sterile cover slips, exposed to 10 μM PARPi for 2 h, and subsequently treated with mock or 3 Gy γ-IR using an X-ray irradiator at 1.225 Gy/min (Kimtron Inc., Woodbury, CT). Immunohistochemistry was performed as previously described [13,14]with slight modification. Please refer to SI materials and methods for details, including antibody used.

Alkaline comet assay

Cells were exposed to ABT-888 and 3 Gy or mock IR and incubated for various times, after which they were prepared and subjected to alkaline comet assay according to the manufacturer's instructions (catalog #4250-050-K; Trevigen). Please refer to SI materials and methods for details.

Clonogenic survival assay

Cell survival was evaluated by the colony formation assay in the HNSCC cell lines following 3 Gy IR and various doses of ABT-888 (1uM-10 μM) as previously described [15]. Briefly, cells were seeded and treated with the indicated doses of ABT 888 (or vehicle) for 2 h, followed by mock or 3 Gy IR following which the plates were left undisturbed. Please refer to SI materials and methods for further details.

Immunoblotting

Cell lysates were prepared using standard protocol. β-Actin levels were analyzed as loading control. Please refer to SI materials and methods for further details.

Statistical analysis

The data were analyzed via analysis of variance (ANOVA) followed by a Bonferroni post test using GraphPad Prism version 4.02 (GraphPad Software, San Diego, CA). Data presented as average ± standard error of mean.

Results

PARPi augments tumor response to radiotherapy

UM-SCC1, UM-SCC5, UM-SCC6, and FaDu cells, which are well characterized and representative squamous cell carcinoma of the head and neck, were utilized in this study [16–19]. PARPi has been shown to be an effective strategy to treat DNA repair deficient cancer. Additionally, PARPi has been combined with other DNA damaging agents with improved outcomes in breast cancer patients [20]. Thus, we hypothesized that the PARPi ABT-888 may increase cytotoxicity of head and neck cancer cells following IR, an integral part of standard therapy for head and neck tumors.

To test this hypothesis, we performed colony forming assays with various doses (1–10 μM) of ABT-888 in combination with IR (3 Gy). These doses of ABT-888 chosen have been previously reported to be within physiologic range [2,21]. IR alone reduced colony forming ability of cells by 30–40% (UM-SCC1: 32%, UM-SCC5: 35%, UM-SCC6: 40%, and FaDu: 30%). As shown in Fig. 1 and consistent with our hypothesis, the addition of IR to PARPi significantly reduced the colony forming ability in a dose-dependent manner (70–95% reduction in cell viability). Interestingly, differential susceptibility to ABT-888 alone was observed. In particular, UM-SCC1 and UM-SCC5 (Fig. 1A and B) cells were exquisitely sensitive to ABT-888 alone (50–75% reduction in cell viability), while UM-SCC6 and FaDu (Fig. 1C and D) were not. These results suggest that the combination of radiotherapy and the PARPi ABT-888 may augment cytotoxicity in head and neck tumors. Additionally, a subset of head and neck cancers may be susceptible to PARPi alone.

Persistent DNA damage with PARPi in head and neck cancer cells

Since IR induces SSB and DSB DNA damage, and because PARP is integral in the repair of DNA single strand breaks (SSBs), we hypothesized that the enhanced cytotoxicity with PARPi and IR may be due to inability of cells to repair DNA damage. To assess this notion, total cellular DNA damage (SSBs and DSBs) following IR and/or ABT-888 was evaluated using the alkaline comet assay. Due to the differential effects of ABT-888 and IR observed (Fig. 1), UM-SCC1 (susceptible to PARPi alone) and UM-SCC6 (not susceptible to PARPi alone) were chosen as representative cell lines for further studies.

As shown in Fig. 2, as expected, the addition of PARPi alone resulted in increased DNA damage, likely due to inhibition of SSB repair. Additionally, similar levels of DNA damage, as evidenced by the mean tail moment, were observed in both PARPi- and vehicle-treated UM-SCC1 and UM-SCC6 (Fig. 2B and C) cells at 15 min after IR. However, at longer time points, increased levels of total DNA damage were observed in irradiated cells treated with PARPi compared with vehicle. Interestingly, at 24 and 48 h following treatment, levels of DNA damage are further increased by the addition of PARPi.

Since the critical cellular DNA lesion in relation to cytotoxic effects are DSBs, we next assessed the effect of PARPi on total DSB damage in head and neck cancer cell lines with and without IR. γ-H2AX foci, well established markers of DNA DSBs [22], were evaluated. As shown in Fig. 2E and F, all cell lines exhibited robust increase in DNA DSBs following IR as demonstrated by increased percentage of cells with γ-H2AX foci. Interestingly the addition of PARPi to IR resulted in increased unresolved DSBs, which have been implicated in initiation of cell death or senescence. These results support a potentiation of DNA damage by PARPi.