Conclusion: These results delineate cGAS-STING induced type 1 interferon release in non-parenchymal cells (NPCs) as a key mediator of IR-induced cancer damage and described a mechanism of innate-immunity-driven pathology, linking cGAS-STING activation with amplification of initial RILD.


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Regulation of DNA Damage Signal-dependent PD-L1 Upregulation In Cancer Cells After Ionizing Irradiation
H. Sato,1 T.B.M. Permata,1,2 W. Gu,3 S. Kakoti,1 T. Oike,1 T. Yasuhara,4,5 T. Nakano,1 T. Ohno,1 and A. Shibata2

Purpose/Objective(s): Studies have suggested that the combination of radiotherapy and anti-PD-1/PD-L1 antibody is promising strategy for cancer treatment. PD-L1 upregulation induced by radiotherapy has been considered as one of the favorable backgrounds. We demonstrated that DNA double-strand break (DSB)-related DNA damage signaling induced by X-ray irradiation upregulates PD-L1 expression. However, the mechanisms underlying PD-L1 upregulation mediated by DNA damage signals after ionizing irradiation have not been completely elucidated. Therefore, we examined the mechanisms underlying PD-L1 upregulation after carbon-ion irradiation with high linear energy transfer (LET) or oxidative DNA stress.

Materials/Methods: Human cancer cells (U2OS, MCF7, HCT116, and H1299) were irradiated by X-ray or carbon-ion beam with high LET (60 KeV/μm) or applied hydrogen peroxide (H2O2). PD-L1 expression was analyzed by immunoblotting, real-time PCR, and flow cytometry. We investigated the involvement of ataxia telangiecisia and Rad3-related protein (ATR) or Chk1 in PD-L1 upregulation after DNA damage using their specific inhibitors. Then, siRNA-depleted cells were analyzed to determine if base excision repair (BER) or single-strand break repair (SSBR) genes are involved in PD-L1 upregulation after DNA damage. PD-L1 distribution on cell surfaces was visualized by super-resolution microscopy. TCGA dataset analysis was used to examine PD-L1 levels in neoplastic samples to confirm reproducibility in clinical samples.

Results: PD-L1 upregulation was observed 48 h after DNA damage induced by 10 Gy carbon-ion irradiation or H2O2 treatment. PD-L1 upregulation was higher after carbon-ion irradiation than that with the same dose of X-rays. PD-L1 upregulations were significantly suppressed after ATR/Chk1 pathway inhibition. Furthermore, PD-L1 upregulation after DNA damage required STAT1 phosphorylation and IRF1 expression. siRNA screening revealed that the majority of BER/SSBR siRNAs augmented PD-L1 upregulation compared with that in control cells after X-ray irradiation. TCGA dataset analysis showed that BER-mutated cancer with high neoantigen production exhibited further increase in PD-L1 expression compared with the BER wild-type cancer with high neoantigen production. Thus, high PD-L1 expression in BER-mutated cancer is not only dependent on the neoantigen pathway but also dependent on the DNA damage signals.

Conclusion: Our study demonstrates that DNA damage signaling from DSB repair, SSBR, and BER after ionizing radiation or oxidative DNA stress regulates the expression of PD-L1 in cancer cells. These findings suggest that the dataset of the expression status of DNA repair genes prior to the treatment would be informative for considering the combination of radiotherapy and immune checkpoint therapy.


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Hyperthermia Induces Radiosensitization and Leads to Reduced Brachyury Levels in Chordoma Cells
A. Saeed1, P. Singh,2 R.S. Malaya,3 J. Mahmood,1 and Z. Vujakovic1

1Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD, 2University of Maryland Baltimore, Baltimore, MD

Purpose/Objective(s): Chordomas are a rare bone malignancy and arise primarily in the sacrum or skull base. Management typically consists of maximal safe resection and adjuvant radiation therapy. Chordomas are inherently radioresistant requiring doses in excess of 70 Gy, which presents a dosimetric challenge given the risk of normal tissue toxicity. Therefore, efforts to induce tumor radiosensitization are needed. We explored the ability of hyperthermia to function as a radiosensitizer, utilizing two human chordoma cell lines. We also examined how hyperthermia impacts brachyury, a transcription factor that serves as a chordoma biomarker and also functions as driver of chordoma tumorigenesis and growth.

Materials/Methods: U-CH2 and MUG-Chor1 (human sacral derived chordoma cell lines) were used for our studies. Hyperthermia’s radiosensitization was assessed by clonogenic survival in colony formation assays. U-CH2 and MUG-Chor1 cells were subjected to hyperthermia (43 degree C for 1 hour) and various doses of radiation (1 – 16 Gy) along with appropriate controls (radiation-alone, hyperthermia-alone, and sham-treated cells). Brachyury protein levels were assessed in U-CH2 and MUG-Chor1 by western blot analysis after cells were subjected to hyperthermia and 4 Gy radiation (and appropriate control treatments) and lysed 16 hours after treatment.

Results: In both U-CH2 and MUG-Chor1 cells, hyperthermia induced radiosensitization as determined by clonogenic survival assay. Specifically, the combination of hyperthermia and radiation treatment reduced colony formation to a greater extent than radiation-alone. Optimal hyperthermia radiosensitization for Mug-Chor1 and U-CH2 cells was observed at 4 Gy. In U-CH2 cells, hyperthermia+irradiation treated cells exhibited a 18% surviving fraction versus 46% for radiation-alone treatment (P < 0.05). In Mug-Chor1 cells, hyperthermia+irradiation treated cells exhibited a 32% surviving fraction versus 85% for radiation-alone treatment (P < 0.05). We next assessed brachyury levels in hyperthermia+irradiation, hyperthermia-alone, radiation-alone, and sham-treated U-CH2 and MUG-Chor1 cells. Western blot analysis demonstrated both cell lines had a marked reduction in brachyury levels in response to hyperthermia-alone, with no further reduction with the addition of radiation.

Conclusion: Hyperthermia functions as a radiosensitizer for chordoma cells base. Additionally, hyperthermia leads to reduced brachyury levels. Collectively, this data raises the possibility that hyperthermia’s radiosensitization may at least in part be due to hyperthermia-induced brachyury reduction. This provides clinical rationale for combining hyperthermia with radiation therapy, especially given clinical pelvic hyperthermia devices are already in use.

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A 18FDG Uptake Gene Signature Predicts Prognosis After Radiotherapy In Breast Cancer
J. Meng,1 L. Zhang,1 W. Shi,1 X. Mei,1 Z. Yang,2 J. Ma,1 X. Yu,2 and X. Guo3

1Department of Radiation Oncology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China, 2Department of Radiation Oncology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

Purpose/Objective(s): Herein, we aimed to identify a predictive gene signature associated with FDG uptake for patients with breast cancer after...