Rapid PTEFb-dependent transcriptional reorganization underpins the glioma adaptive response to radiotherapy

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BACKGROUND

Pediatric high-grade gliomas are highly lethal malignancies for which radiation remains the only uniformly accepted standard of care. As such, mechanisms to further sensitize these tumors to radiotherapy, augmenting the depth and duration of clinical response, remain attractive strategies for clinical practice. Dynamic regulation of gene expression is fundamental for cellular adaptation to exogenous stressors. Promoter proximal pause-release of RNA polymerase II (Pol II) is a conserved regulatory mechanism for synchronous transcriptional induction in response to heat shock, but this pro-survival role has not been examined in the applied context of cancer therapy. Here, we use model systems of pediatric high-grade glioma to characterize transcriptional mechanisms underpinning the adaptive response to ionizing radiation (IR) in order to define specific regulators or cofactors amenable to therapeutic disruption.

METHODS

- We performed RNA-Seq following radiotherapy in the presence or absence of CDK9 in patient derived DIPG cell lines to determine consequent impact on net transcriptional output
- We assessed cell death via caspase 3/7 and live cell imaging in HGG cultures treated with CDK9, IR or combination
- Clonogenic survival was assessed by CFA in HGG cultures treated with CDK9, IR or combination
- IC50s were obtained for neoplastic and normal cell lines following sustained or intermittent CDK9 inhibition
- Coculture systems of DIPG cells and astrocytes were treated with IR and intermittent AZD and imaged using a live cell imaging system
- We used an orthotopic PDX model of DIPG and DMG, conformal fractionated radiation, bioluminescent imaging, and MRI to assess probability of survival

RESULTS

Fig 1. A. Western blot analysis of phosphorylation following radiotherapy in the presence or absence of CDK9 versus DMG treated control over six and twelve hours. B. Heat map and C. quantification of genes up or down regulated +/- LFC 1.5 following radiotherapy in the presence or absence of CDK9. D. Specific upregulated DDR genes and their LFC. (A: **p<0.01, *p<0.05 vs control; B: Heatmap; C: *p<0.01, **p<0.05 vs control) (n=3)

Fig 2. A. Quantified caspase 3/7 activity (left) and representative fluorescent live-cell imaging (right) of HGG cultures treated with IR, CDK9, or combination. B. Colony focus assay images (left) and quantification (right) of HGG cultures treated with AZD4573, IR, or combination. (A: *p<0.05, **p<0.01 vs IR alone, n=3; B: *p<0.05 vs compound vs IR alone, n=3)

Fig 3. A. AZD4573 IC50 after 3-day exposure in respective cell lines. B. Western blot analysis of p-Pol II (Ser 2) and MCL1 after indicated exposure times to 50 nM AZD4573. C. Caspase 3/7 activity over time following fixed dose of AZD4573. D. IC50 and comparison as in (a) but measured 3 days after a single 8-hour drug exposure followed by drug washout. E. Quantification of relative ratio of co-cultured DIPG cells (HSJUD-DIPG007) and normal astrocytes (rHAA-nERT) following fractionated radiotherapy and intermittent AZD4573 treatment at day 10. (A: *p<0.05 vs control; B: *p<0.05 vs control; C: *p<0.01, **p<0.001 vs control) (n=3)

Fig 4. A. Survival analysis and B. bioluminescent flux values of Zotarolimus, IR, and combination treated cohorts versus vehicle control. C. Axial T2-weighted turboRARE MRI sequences of selected mice from IR and combination treated cohorts. (A: *p<0.05 vs vehicle control, n=6; B: *p<0.05, **p<0.01 vs vehicle control, n=6; C: *p<0.05 vs vehicle control, n=6)

CONCLUSIONS

We show here that Pol II induction is facilitated within hours of exposure to therapeutic ionizing radiation. Concurrent inhibition of CDK9 imparts a transcription elongation defect, abrogating canonical adaptive programs such as DNA damage repair and cell cycle regulation. This combination demonstrates a potent, synergistic therapeutic potential diagnostic of glioma subtype, leading to a marked induction of tumor cell apoptosis and prolongation of xenograft survival. Our data here suggests that not only selectivity, but timing and duration of dose exposure is critical in achieving a tolerable therapeutic index. These studies reveal a central role for PTEFb underpinning the early adaptive response to radiotherapy, opening new avenues for combinatorial treatment in these lethal malignancies.

FUTURE DIRECTIONS

CDK9 has been recognized as a promising target for cancer therapy for more than a decade, prompting the formulation of numerous inhibitory compounds now in various stages of preclinical and clinical development. The data here will serve as a basis for first-in-pediatrics clinical trial currently in development.

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