Effect of Stereotactic Radiosurgery on Non-small Cell Lung Cancer Brain Metastasis: Continued Correlative Radiobiologic Analysis of DNA and RNA Genomic Profiles from Phase-II Clinical Trial NCT03398694

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ABSTRACT

BACKGROUND: Stereotactic radiosurgery (SRS) is an increasingly common modality used with or without surgery for the treatment of brain metastases (BM). However, the molecular and genomic effects of SRS on tumors *in vivo* is unknown.

METHODS: Patients were treated with SRS prior to surgery as per clinical trial protocol (NCT03398694). Resected tumor was divided into two groups: 'center' and 'periphery' with respect to the center of SRS treatment. Tissue were analyzed by DNA and RNA sequencing and compared between the two groups as well as to non-radiated control tumor tissue.

RESULTS: DNA analysis showed at the individual level, matched comparison of SRS samples from the center or periphery of the same tumor had total mutational burden differences. In terms of RNA analysis, there were no differentially expressed genes between center and peripheral radiated BM, but there were 62 and 192 differentially expressed genes between the center as well as peripheral tumor and non-radiated control, respectively. At an individual level in matched center and peripheral tumor of SRS-treated patients revealed an average of 16641 differentially expressed genes. Comparing total number of up- and downregulated genes with total SNP and Indel mutations of matched patient samples, we noted that in patients with higher mutational burdens in peripheral tumors as compared to center, there was a much higher number of upregulated genes in center as compared to matched peripheral tumor. Reciprocally, we also noted when mutation burden was higher in center tumor, total number of genes that were either up- or downregulated were roughly about the same. Pooled analysis revealed significant upregulation of oncogenes, such as TP63, LEF1, and RECQL4, in the group treated with radiation. DO enrichment analysis also reveal pathways related to non-small cell lung carcinoma and lung carcinoma significantly altered in radiation cohort.

CONCLUSION: In summary, this study demonstrates that SRS alters the molecular and genomic profile of non-small cell lung cancer brain metastasis. It results in altered expression of oncogenes and pathways related to lung cancer. Additionally, by sampling the tumor at the center and periphery, we observed that there is differential effects of the dose gradient on the cellular and molecular response to ionizing radiation.

INTRODUCTION

Stereotactic radiosurgery (SRS) is an increasingly common modality used with or without surgery for the treatment brain metastases (BM). While post-resection cavity SRS has roughly equivalent local control compared to post-resection WBRT, several factors suggest that pre-operative SRS has advantages over post-operative SRS.[1] Radiosurgery is known to have a sharp penumbra. Targeting a resection cavity is imprecise and there may be microscopic spread of disease outside of the contoured volume as a result of post-surgical disruption of blood brain barrier. Another advantage of pre-operative SRS is a theoretical increased response to radiation due to intact vasculature and greater peri-tumoral oxygen content.

Additionally, there are no published reports of the *in vivo* effects of SRS in the immediate post-procedure setting in human metastatic brain tumor, and pre-operative SRS treatment paradigm provides a unique opportunity to evaluate radiobiologic effects of ablative doses of radiation with 72 hours post treatment. Tissue radiosensitivity is under genetic control.[2] As such, it is important to investigate the evolution of the genomic profile under the pressure of radiation leading to the eradication verses resistance/recurrence, especially in correlation to patient's local control and distant metastasis.

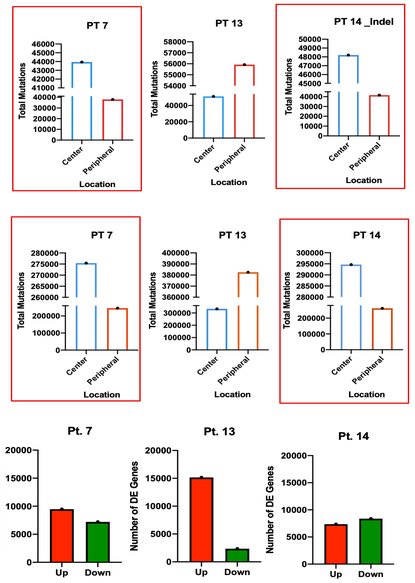
METHODS

Patient selection: This study was approved by Indiana University Institutional IRB. Patients with 1-4 brain metastases on a diagnostic brain MRI or CT were identified as potential study subject per the recommendation of surgeons, medical oncologists, and/or radiation oncologist. Patients were selected based on pre-set inclusion and exclusion criteria.[3] Non-radiated, small lung cell metastasis controls were also included in this study

Pre-operative SRS and tumor sample selection: GK-SRS was delivered 1-3 days prior to surgical resection according to RTOG-9005 dosing criteria with the exception that the largest lesion diameter treated with 15 Gy was 5 cm. All the lesions were treated with SRS and the largest and/or symptomatic lesions were resected. The resected tumor specimens were divided into two groups: 'center' and 'periphery' with respect to the center of SRS treatment with periphery within 50% isodose line.

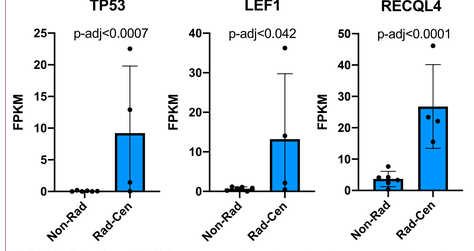
Genome sequencing: DNA and RNA was isolated using the Qiagen AllPrep DNA/RNA Mini Kit (Cat.No:80204) according to manufacturer's protocol. Briefly, tissue was lysed and homogenized, and DNA/RNA was purified using the AllPrep DNA/RNA spin column. DNA and RNA was submitted to Novagene Inc. for sequencing according to company protocol. ANOVA was used to determine if variant frequencies between treatment conditions was statistically significant.

RESULTS 1: Individual patients' SNP and Indel total mutation number correlate with DE gene number



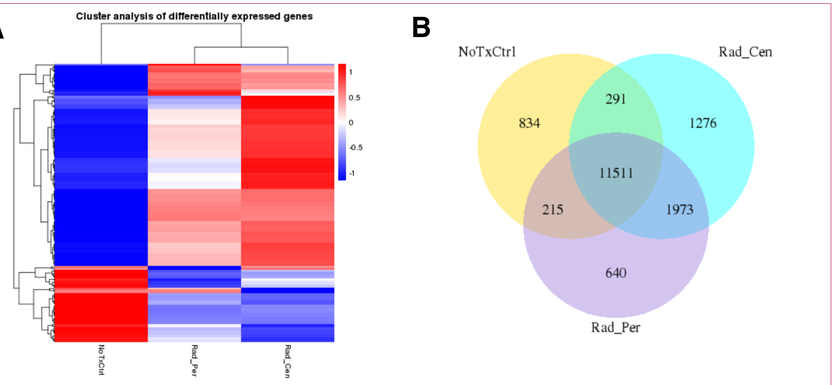
RESULT 1: A) 4 out of 5 (80%) patients showed increased total number of Indels in center versus periphery locations. **B)** 3 out of 5 (60%) patients showed increased number of SNPs in center versus periphery locations. **C)** Total number of DE genes of matched patient samples in center vs periphery tissues.

RESULTS 3: Selected oncogenes are upregulated in radiated tissues



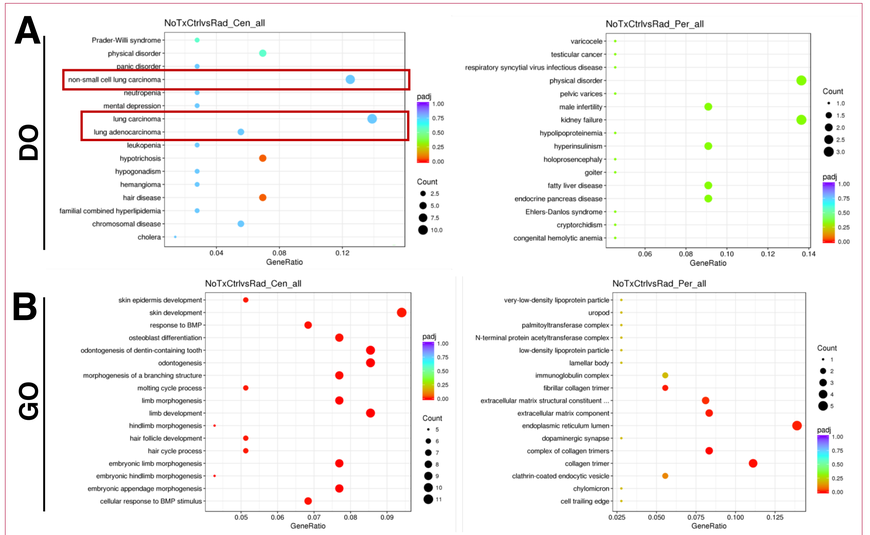
RESULTS 3: FPKM expression levels in non-radiated vs radiated center tissue with p-adj values.

RESULTS 2: RNA sequencing reveals genomic difference in radiated versus non-radiated tissues



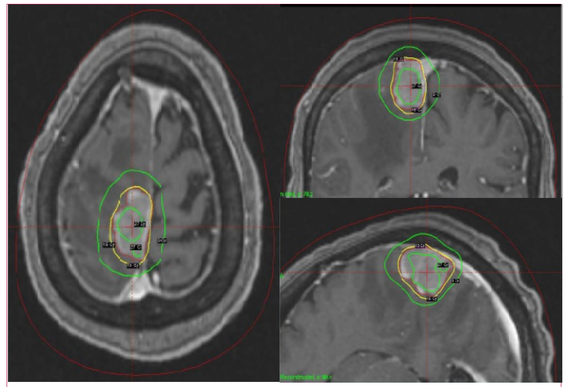
RESULT 2: A) Heatmap analysis of non-radiated vs radiated center vs radiated peripheral tissue. **B)** Venn diagram of total shared or unique differentially expressed genes.

RESULTS 4: Pathway analysis reveals radiation-induced differential expression in radiated vs non-radiated NSCLC brain metastasis



RESULT 4: A) Disease Ontology (DO) pathways analysis. B. Gene Ontology (GO) pathway analysis.

METHODS CONT.



Gamma knife plan for one of the patients on the pre-op SRS protocol

- Small green circle: 150% IDL
- Yellow circle: 100% IDL
- Big green circle: 50% IDL

DISCUSSION

Our study has shown that tumor undergoes molecular changes in response to radiation treatment. Additionally, individual matched patient analysis reveals patient variability in response to radiation treatment and gene expression patterns differ depending on mutation burdens.

Pathway analysis also reveal significant differences in the radiated vs non-radiated cohort and that pathways differentially expressed are not shared in center and peripheral radiated tissue, suggesting a differential response to radiation in a dose-dependent manner. Interestingly, oncogenes are also upregulated in radiated center tissue as compared to non-radiated controls.

CONCLUSION

Our study has shown that pre-operative stereotaxic radiosurgery induces mutational and genomic burdens in NSCLC brain metastasis. Our study also demonstrates the power of using individualized matched patient sample analysis to determine patient-to-patient variability response to radiotherapy. Future studies will focus on additional genomic analysis, immunohistochemistry, and histopathology analysis.

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ACKNOWLEDGEMENTS

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