Biology Abstracts

Presentation Number: 115

Using Mouse Genetics to Dissect the Radiobiology of SBRT: Tumor Cells, Not Endothelial Cells, Regulate Local Control

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Purpose/Objective(s): Advances in treatment planning and delivery have made it possible to safely deliver a small number of high radiation doses to tumors through a treatment modality termed stereotactic body radiation therapy (SBRT). SBRT has been shown to increase local control of many tumors, leading some to propose that high dose radiation therapy may engage new mechanisms of tumor eradication. Human cancers develop in a complex environment composed of blood vessels, fibroblasts, and immune cells. However, it remains controversial whether stromal cells, such as endothelial cells, or tumor cells are the critical targets that regulate tumor eradication by SBRT. Genetically engineered mouse models of cancer develop within a native tumor microenvironment in immunocompetent mice and may more faithfully recapitulate the tumor microenvironment of human cancer than transplanted models. To determine the critical target(s) in primary tumors that mediate local control by SBRT, we used dual recombinase technology to selectively manipulate the radiosensitivity of endothelial cells or tumors cells in primary mouse soft tissue sarcomas.

Materials/Methods: Using novel dual recombinase technology, we initiated primary soft tissue sarcomas in FSF-Kras\textsuperscript{G12D}; p53\textsuperscript{FRT/FRT} mice by intramuscular injection of an adenovirus expressing FlpO recombinase and deleted floxed alleles of Bax or Atm in endothelial cells using the tissue-specific Cre-driver VE-Cadherin-Cre. We also deleted Atm in tumor cells by intramuscular injection of 4-hydroxytamoxifen into Pax7-CreER; LSL-Kras\textsuperscript{G12D}; p53\textsuperscript{FL/FL}; Atm\textsuperscript{FL/FL} mice. Following tumor development, mice were treated with 20 Gy or 50 Gy focal sarcoma irradiation using an X-RAD 225Cx small animal image-guided irradiator, and sarcoma growth delay and local control were monitored by caliper measurement.

Results: Deletion of Bax in endothelial cells did not affect endothelial cell death or tumor response to radiation therapy. Deletion of Atm in endothelial cells increased radiation-induced cell death of tumor endothelial cells and prolonged tumor growth delay following a non-curative dose of radiation. However, following a curative dose of radiation, Atm deletion in endothelial cells did not affect growth delay and failed to increase local control. In contrast, deletion of Atm specifically in tumor cells increased local control of primary tumors by radiation therapy.

Conclusions: Tumor cells rather than endothelial cells are the critical targets that regulate primary tumor eradication by SBRT.


Presentation Number: 219

Integrative Radiogenomic Profiling Identifies BRAF Mutations as Novel Radiotherapeutic Targets in Adenocarcinomas of the Lung

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Purpose/Objective(s): Patients with non-small cell lung cancer (NSCLC) display a wide spectrum of oncologic outcomes, suggesting significant underlying biologic diversity. Despite two notable exceptions in cases of
EGFR mutations and ALK rearrangements, current therapeutic management is largely homogeneous for a given stage. To advance genotype-directed radiotherapy in NSCLC, we sought to identify genetic determinants of radioresistance by leveraging cancer genomic data with a recently developed high-throughput platform for measuring radiation survival.

Materials/Methods: To adequately represent the biologic spectrum of lung cancer and maximize power to detect clinically relevant genotypes, we profiled 104 lung cancer cell lines, including 89 NSCLC and 15 small cell lung cancer (SCLC) lines. We used our recently validated high-throughput proliferation assay to measure survival. Survival curve analysis permitted quantitative assessment of radiosensitivity. Genomic correlates of radiosensitivity were explored by analyzing Oncomap data from the Cancer Cell Line Encyclopedia, the COSMIC database of the Cancer Genome Project, and The Cancer Genome Atlas.

Results: Radiation survival across lineages reflected clinical experience and the literature regarding differential radiation response, inasmuch as lung squamous cell carcinoma and adenocarcinoma (ACA) had similar radiosensitivity, whereas SCLC and carcinoid were, respectively, more and less radiosensitive. Importantly, radiosensitivity varied more within a lineage than across lineages, with a 6-fold difference in integral survival among ACA lines. Correlation with cancer genomic data revealed clustering of BRAF mutations among the most resistant ACA lines (p = 0.035). Analysis by genotype revealed BRAF-mutant ACA lines to be significantly more resistant than BRAF wild-type ACA lines (p = 0.023). All mutations identified in our analysis (G469A, G466V, and L597V) have been confirmed in lung ACA tissue samples. Moreover, these mutations are all located in the highly conserved kinase domain, with the majority known to enhance kinase activity in melanoma in a manner analogous to the well-known BRAF V600E mutation.

Conclusions: Integration of high-throughput radiation survival profiling with large-scale cancer genomic data suggests BRAF mutations are associated with radiation resistance in lung ACA. Our analysis nominates BRAF inhibitors, which are commercially available, as radiosensitizers in select BRAF-mutant lung ACA. Further investigation has the potential to yield an additional genotype-directed therapy that could impact up to 7% of patients with lung ACA, a prevalence comparable to that of ALK rearrangements (4%) or EGFR mutations (10%).


Presentation Number: 70

Maternal Embryonic Leucine Zipper Kinase (MELK): A Novel Target for Radiosensitization That is Independently Prognostic in Triple-Negative Breast Cancers

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Purpose/Objective(s): Increased rates of locoregional recurrence have been observed in TNBC despite the use of RT. Therefore approaches that result in radiosensitization in TNBC are critically needed. Our previous work identified one such novel molecular target as MELK and we sought to investigate the impact of MELK expression on radiation response and patient outcomes.

Materials/Methods: Using data from gene expression microarrays, we interrogated the expression of MELK in 2,061 human breast tumor samples and 51 breast cancer cell lines. Overexpression of MELK protein in TN and basal-like cancers was confirmed and clonogenic survival assays were used to quantify the degree of radiosensitivity of breast cancer cell lines at baseline and after MELK inhibition. We measured in vivo tumor growth and doubling time using xenografts in mice with vehicle control, RT alone, inhibitor or knock-down
alone, or combination RT+MELK inhibition groups (16-20 tumors/group). Kaplan-Meier analysis was performed to determine the clinical impact of MELK on local control and survival. A Cox proportional hazards model was constructed to identify potential factors of survival. The initial multivariate model contained all variables independently associated with LRF-survival in univariate analyses. A backward selection procedure, using p≤0.05 as the criterion for inclusion, was then implemented to build the final model.

**Results:** MELK expression was significantly elevated in human TNBC, including radiation resistant tumors (p-value 7.5e-21). MELK protein and RNA expression were induced by ionizing radiation (5.6-7.5 fold at 72 hours, p-value <0.01). MELK expression was significantly correlated with radioresistance in 21 breast cancer cell lines (R: 0.62, p-value 0.003). Inhibition of MELK using both siRNA and small molecule inhibitors induced radiation sensitivity in vitro with an enhancement ratio (ER) of 1.5-1.6 through impaired dsDNA repair and cell cycle arrest mechanisms. MELK inhibition either with gene knock-down or small molecule inhibitors significantly radiosensitized TNBC xenografts in mouse models and markedly delayed tumor doubling time and tumor growth (median tumor doubling time 7.95 days for RT alone vs. 29.1 days for MELK inhibition + RT, p-value <0.0001). Analyses of patients with breast cancer showed that patients whose tumors have high expression of MELK had markedly higher rates of LR after RT and an overall poorer prognosis than patients with low expression of MELK (HR for LR 1.89-2.23, p-value <0.001; HR for OS 1.46-3.3; p-value <0.001 in 3 independent datasets). In multivariate analysis, only MELK expression and grade were significantly associated with worse LRF survival with a HR of 1.35 (95% CI 1.05-1.72, p-value <0.01).

**Conclusions:** Our results support the rationale for developing clinical strategies to inhibit MELK as a novel target in TNBC.

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**Presentation Number:** 189

**Somatic ERCC2 Mutations Confer Cisplatin Sensitivity in Muscle-Invasive Urothelial Cancer**

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**Purpose/Objective(s):** To discover and validate genetic predictors of cisplatin response in muscle-invasive urothelial carcinoma.  

**Materials/Methods:** We performed whole exome sequencing of pre-treatment tumor and germline DNA from 51 patients with muscle invasive urothelial carcinoma who received neoadjuvant cisplatin-based chemotherapy followed by cystectomy. The cohort consisted of 26 “responders” with pT0/pTis disease on pathologic examination at cystectomy, and 25 “non-responders” with ≥T2 disease. Somatic mutations and short insertions/deletions were identified using validated techniques, and additional computational methods were employed to identify mutations occurring preferentially in cisplatin responders. Functional validation of significantly enriched mutations was performed using cellular and biochemical techniques.

**Results:** Statistical analysis nominated five known cancer genes as significantly mutated in tumor samples. ERCC2, a nucleotide excision repair (NER) gene, was the only gene for which mutations were significantly enriched in the cisplatin responders compared to non-responders. Somatic ERCC2 missense mutations were observed in 10 of 26 (38.5%) responders and 0 of 25 (0%) non-responders (q<0.01). ERCC2 mutations were also more frequent in the responder cohort than in a separate unselected urothelial cancer cohort from the Cancer Genome Atlas (38.5% vs 12%; p<0.01). The identified ERCC2 mutations are present within or adjacent to conserved helicase sequence motifs, and structure-based modeling predicts the mutations to cluster near a functionally-relevant interface between helicase domains. Expression of a subset of the identified mutants in an ERCC2-deficient cell line failed to rescue cisplatin or UV sensitivity compared to wild-type ERCC2. The ERCC2 mutant tumors also had a higher background mutation rate than ERCC2 wild-type tumors (14.7 vs 5.1 mutations/megabase; p<0.01), and ERCC2 mutant cell lines displayed an increased number of chromosomal...
aberrations following cisplatin exposure, indicative of increased global genomic instability.

**Conclusions:** Somatic ERCC2 mutations are associated with cisplatin response in muscle invasive urothelial carcinoma. ERCC2 mutations may confer increased cisplatin sensitivity through loss of normal NER capacity following exposure to DNA damaging agents. These results may inform the use of cisplatin-containing regimens in muscle invasive urothelial carcinoma, and potentially other ERCC2-mutated tumors.


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**Presentation Number:** PL-01

**Role of Radiotherapy in Inducing Antigen Specific Anti-Tumor Immune Responses When Combined With Anti-PD1 Checkpoint Blockade: Mechanism and Clinical Implications**

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**Purpose/Objective(s):** Immunotherapy with anti-CTLA-4 and anti-PD1 antibodies (Ab) has gained significant interest due to activity in multiple tumor types and high rates of systemic control in metastatic melanoma. Pre-clinical studies and case reports have described potential synergy between radiotherapy (RT) and immunotherapy (IT); however, the direct role that RT may play in augmenting anti-tumor immune responses remains unclear. Here we report a mechanistic role for RT in inducing antigen-specific immune responses when combined with anti-PD1 Ab in melanoma and breast carcinoma.

**Materials/Methods:** Stereotactic image-guided RT was delivered to B16-OVA and 4T1-HA flank tumors via the small animal radiation research platform (SARRP). Mice received three IP injections of 200ug anti-PD1 and/or anti-CTLA-4 Ab before, during, and after RT. Cell surface markers and development of antigen-specific immune responses was analyzed by flow cytometry on LSR II. Adoptive transfer studies were performed to validate protective immunity and in-vitro studies elucidated a molecular mechanism of action.

**Results:** Stereotactic RT modified the immunophenotype by increasing the surface expression of tumor associated antigens, MHC, and Fas. RT of B16-OVA enhanced tumor associated antigen presentation in-vitro and in the draining lymph node (DLN) in-vivo. Additionally, hypofractionated RT of 18Gy x1 or 7Gy x3 increased the proliferation and activation of antigen specific CD8 T-cells in the DLN (IFN-gamma positive: Mock 20.8 +/- 3.6% vs 18Gy 45.6 +/- 1.9%, p<0.01). We observed significant increases in the development of endogenous SIINFEKL Pentamer positive anti-tumor immune responses when RT was combined with anti-PD1 Ab (RT 0.6% vs RT + anti-PD1 1.3%, p<0.05). Furthermore, combination therapy resulted in primary tumor control, increased survival of treated mice, and elicited the abscopal effect (B16-OVA: IT 372 +/- 138cc, RT 121 +/- 43cc, RT+IT 57 +/- 31 cc*, p<0.05; 4T1-HA: IT 663 +/- 23cc, RT 351 +/- 150cc, RT+IT 99 +/- 51cc*, p<0.05). Mechanistically we identified that RT upregulates specific chemokine receptors which may drive development of antigen specific immune responses.

**Conclusions:** RT altered the immunophenotype of tumor cells and enhanced tumor associated antigen presentation. Combination RT + IT resulted in significantly increased antigen-specific immune responses, primary tumor control, and abscopal effects outside of the radiation field. These data provide critical evidence supporting the ability of stereotactic RT to induce anti-tumor immune responses and augment the efficacy of anti-PD1 checkpoint blockade in melanoma and breast carcinoma. These findings have important implications for the novel treatment paradigm of RT combined with IT and provide mechanistic rational for clinical trials of combined therapy in the definitive and metastatic setting.

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