

**1100 The Effect of Proteasome Inhibitor Velcade on Tumor Microenvironment**

X. Sun, F. He, E. Ackerstaff, L. Xing, A. Minami, S. Carlin, C. Ling, J. Koutcher, G. Li

Memorial Sloan-Kettering Cancer Center, New York, NY

**Purpose/Objective(s):** It has been reported that the therapeutic effect of the proteasome inhibitor Velcade is due to its selective interference in the hypoxia pathway. We studied the effect of Velcade on tumor microenvironment and examined the underlying molecular mechanisms.

**Materials/Methods:** We generated a human colorectal cancer xenograft model (#C53) in which the hypoxia-inducible dual reporter fusion gene (HSV1-TK and eGFP) was under the control of hypoxia-response-element (HRE). *In vitro*, #C53 cells were treated with Velcade in normoxic and hypoxic conditions, and the following assays were performed in comparison with controls: eGFP (flow cytometry), CA9 (Western blot), VEGF (ELISA), and TK activity (trapping of the marker substrate <sup>14</sup>C-FIAU). *In vivo*, #C53 xenografts were treated with Velcade and various assays performed, including (1) Dynamic contrast-enhanced (DCE) MRI pre- and post-treatment; (2) dual hypoxia marker (pimonidazole and EF5) administration pre- and post-treatment; (3) fluorescence microscopy of Hoechst 33342 (perfusion), eGFP, HIF-1 $\alpha$ , and CA9; and (4) plasma VEGF level (ELISA), where applicable data from control and treated tumors were compared.

**Results:** In both *in vitro* and *in vivo* experiments, Velcade treatment increased the level of HIF1 $\alpha$ , but decreased those of hypoxia-induced eGFP, TK, CA9, and VEGF. Interestingly, in the dual hypoxia marker study there were significant EF5-positive regions that did not co-localize with pimonidazole-positive regions, suggesting *de novo* hypoxia and perhaps another novel effect of Velcade on tumor microvasculature. Consistent with these results, DCE MRI demonstrated decreased global tumor blood flow with Velcade treatment.

**Conclusions:** Our data suggest that Velcade suppresses the hypoxia response by disrupting the HIF1 transcriptional activity. In addition, our results suggest a novel function of Velcade in modifying the tumor microenvironment and decreasing tumor perfusion as noninvasively detected by DCE MRI.

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**1101 The Unique Microenvironments of Spontaneous Tumors Differentially Sensitize Them to Radiation**

M. T. Spiotto, A. Bahn, H. Cao, Q. Le, A. C. Koong

Stanford University Cancer Center, Palo Alto, CA

**Purpose/Objective(s):** The tumor microenvironment remains a poorly characterized aspect of cancer biology. Yet, it remains unclear how variable the microenvironment is between similar tumors and how these differences affect responses to therapy. One putative marker for the tumor microenvironment is endoplasmic reticulum (ER) stress which can be triggered within cancer cells by severe hypoxia or nutrient deprivation. Here, we developed breast cancer prone transgenic mice that reported ER stress as surrogate marker for the microenvironment in primary tumors.

**Materials/Methods:** We generated an X-box binding protein 1 (XBP1)-luciferase (XBP1-luc) transgenic mouse that was capable of reporting ER stress. We bred XBP1-luc mice to breast cancer prone Tag mice (FVB/N-Tg[MMTV-PyVT]634 Mul/J) generating double transgenic mice in order to monitor ER stress in primary mammary tumors. We imaged XBP1-luc activity in these tumors and correlated it to glucose avidity and radiosensitivity. Two-sided independent *t* test without equal variance was performed to analyze the results of *in vivo* bioluminescence and tumor growth timepoints. The ANOVA tests were performed to analyze correlations of *in vivo* bioluminescence with *in vitro* luciferase activity and *in vivo* uptake of fluorescently-labeled 2-deoxy-d-glucose.

**Results:** Tag-Luc mice possessed XBP1-luc bioluminescent signal that was  $9.36 \pm 0.08$ -fold greater than the background skin and significantly greater than the primary tumors arising in Tag mice ( $p = 2.99 \times 10^{-15}$ ). *In vivo* bioluminescence signal significantly correlated with *in vitro* luciferase activity of these primary tumors ( $p = 7.73 \times 10^{-5}$ ). Individual primary tumors within the same mouse had higher or lower levels of XBP1-luc activity that were unique to that primary tumor and not maintained by serial *in vivo* passages. This heterogeneity of XBP1-luc activity in primary tumors inversely correlated with their glucose avidity ( $p = 0.0019$ ). Finally, primary tumors with higher levels of XBP1-luc activity had more nonviable cells after 5 Gy of irradiation ( $p = 0.00005$ ). Compared to radiation alone, tumors in mice treated with bortezomib to increase ER stress regressed more after irradiation ( $p = 0.03$ ).

**Conclusions:** The ER stress caused by the tumor microenvironment is distinct between primary tumors and is not recapitulated in the transplant setting. These differences reflected distinct glucose avidities between tumors and correlated with increased radiosensitivity. These results provide a basis for monitoring and modulating aspects of ER stress in the tumor microenvironment to increase radiosensitivity and tumor cure.

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**1102 In Vivo shRNA to Study the Molecular Mechanisms of Radiation Response of Primary Cancers in Mice**

A. Ghafoori<sup>1</sup>, C. Lee<sup>1</sup>, B. Perez<sup>1</sup>, S. Johnston<sup>1</sup>, R. Rodrigues<sup>1</sup>, C. Badae<sup>1</sup>, Y. Kim<sup>2</sup>, S. Lowe<sup>3</sup>, D. G. Kirsch<sup>1</sup>

<sup>1</sup>Duke University Medical Center, Durham, NC, <sup>2</sup>North Carolina State University, Raleigh, NC, <sup>3</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

**Purpose/Objective(s):** Genetically-engineered mouse models (GEMMs) with primary cancers are an elegant model system for studying radiation biology because these tumors faithfully recapitulate key aspects of human tumors. We are studying mechanisms of tumor response to radiation therapy (RT) using GEMMs for non-small-cell lung cancer (NSCLC) and soft tissue sarcoma (STS).

Recent developments with the use of short hairpin RNA (shRNA) technology have raised the possibility of “knocking down” the expression of specific genes *in vivo* to define the role they play in the response of tumors to RT. In the present study, we describe the integration of *in vivo* shRNAs for conditionally knocking down expression of the tumor suppressor gene p53 in primary lung cancers and soft tissue sarcomas in mice.

**Materials/Methods:** The GEMMs of primary NSCLC and primary lower extremity STS were generated using the Cre-lox recombination system. These mice also expressed a p53-directed shRNA that was regulated by a tetracycline-dependent transactivator (rtTA), which binds to and activates the tetracycline response element (TRE) in the presence of doxycycline. Therefore, doxycycline can induce transcription of shRNAs targeting p53 for knock-down. Mice with NSCLC or STS were treated with or without doxycycline. Tumors were followed with micro-CT for mice with NSCLC and clinically for mice with lower extremity STS. Individual tumors were excised from the lungs or lower extremities and processed for histology and RT-PCR for p53-directed shRNA expression. Additionally, primary cell lines were generated from the excised STS primary tumors. The RNA was isolated from STS tumor cells treated with doxycycline for 48 hours and RT-PCR was performed for p53-directed shRNAs.

**Results:** Tumor development and progression were confirmed with micro-CT (NSCLC) or clinically (STS). Quantitative real-time RT-PCR analysis of the excised tumor specimens revealed expression of p53-directed shRNAs both in NSCLC and STS from mice receiving doxycycline. Mice with no doxycycline had tumors with undetectable levels of shRNAs. Primary cell lines generated from excised primary STS also expressed p53-directed shRNAs, but only after treatment with doxycycline. Histologic analysis by a veterinary cancer pathologist blinded to treatment revealed that lung tumors were of higher grade if they were obtained from mice treated with doxycycline.

**Conclusions:** These findings demonstrate that we can successfully express an shRNA to p53 *in vivo* in both primary NSCLC and STS in mice. Lung tumors with p53-directed shRNA expression were more aggressive by histology, demonstrating functional knock-down of p53. This system will allow us to knock down specific genes *in vivo* and will be a powerful tool to test their role in the response of primary cancers to RT.

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### 1103 Treatment with R-Spondin 1 and Toll-like Receptor 9 Ligands Improve the Therapeutic Ratio of Abdominal Irradiation

P. Bhanja<sup>1</sup>, S. Saha<sup>1</sup>, L. Liu<sup>1</sup>, R. Kabarriti<sup>1</sup>, H. Zhang<sup>1</sup>, N. Deb<sup>1,2</sup>, M. Garg<sup>1,2</sup>, S. Kalnicki<sup>1,2</sup>, A. Alfieri<sup>1,2</sup>, C. Guha<sup>1,2</sup>

<sup>1</sup>Albert Einstein College of Medicine, Bronx, NY, <sup>2</sup>Montefiore Medical Center, Bronx, NY

**Purpose/Objective(s):** Radiation therapy (RT) of abdominal malignancies is limited by the induction of potentially lethal radiation-induced gastrointestinal syndrome (RIGS), which is characterized by cell death of intestinal crypt stem and endothelial cells resulting in villus denudation and symptoms ranging from diarrhea, electrolyte imbalance, weight loss/sepsis, and death. Previous studies in our laboratory have demonstrated that RIGS can be prevented by systemic administration of R-spondin 1, an intestinal stem cell growth factor and/or immunomodulatory oligonucleotides (IMO), a Toll-like receptor 9 ligand (TLR-9); both of which induces proliferation and radioresistance of the intestinal stem cell compartment. This study was undertaken to examine whether R-spondin 1 and IMO can be used as a radioprotectant of the intestine without conferring radioresistance of tumor.

**Materials/Methods:** Male BALB/c mice with palpable CT26 colon tumors in abdominal wall were treated with a 14 Gy single dose of whole abdominal RT (AIR). Experimental cohorts included: (1) AdRspo1 - intravenous injection of adenovirus expressing recombinant huRspo1 (1,011 particles at Days 3 and 1 prior to AIR); (2) AdLacZ controls; (3) IMO (1mg/kg sc), 1 hour prior to AIR; and (4) PBS alone. Animals were observed for tumor growth and survival. Moribund animals were sacrificed and intestine and tumor tissues were harvested for histology.

**Results:** Although, AIR reduced tumor growth significantly, all animals died within 15 days, secondary to RIGS. The AdRspo1 and IMO treatment significantly increased the survival of mice treated with AIR ( $p < 0.0001$ , log-rank test Kaplan-Meier) with 70% and 60% of mice surviving 28 days after AIR+AdRspo1 and AIR+IMO, respectively. In addition, AIR+IMO treatment-induced tumor-specific immune response and significantly reduced tumor growth, compared to mice receiving AIR alone (150 + 7.25 mm<sup>3</sup>, AIR vs. 85 + 4.25 mm<sup>3</sup>, AIR+IMO,  $p < 0.003$ , 2-tailed *t* test). Histological analysis and xylose absorption test demonstrated significant structural and functional regeneration of the intestine in irradiated animals, receiving AdRspo1 and IMO treatment. Immunohistochemical analysis demonstrated the translocation of  $\beta$ -catenin from the cytosol to nucleus and upregulation of Wnt- $\beta$ -catenin target genes in AdRspo1-treated mice, as compared to AdLacZ-treated mice, indicating that Rspo1 confers radioprotection of RIGS by stimulating the Wnt pathway.

**Conclusions:** Systemic treatment with Rspo1 and IMO improved the survival of mice receiving AIR by preventing RIGS without conferring tumor radioprotection. The IMO further enhanced the tumoricidal effects of AIR. Thus, systemic administration of R-spondin 1 and TLR9 ligands could improve the therapeutic ratio of RT in abdominal malignancies.

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### 1104 The Role of Nitric Oxide Synthase and Heme Oxygenase I in Radiation-induced Lung Injury

P. R. Graves, T. Van Meter, E. Rosenberg, R. Mikkelsen

Virginia Commonwealth University, Richmond, VA

**Purpose/Objective(s):** Radiotherapy (RT) is the main treatment modality for inoperable lung cancer but is limited by the low tolerance of the surrounding normal lung tissue. Previous studies demonstrated that ionizing radiation (IR) stimulates formation of nitric oxide (NO) and 3-nitrotyrosine in the lung, the latter of which is associated with lung injury. In contrast, radiation also