Purpose/Objective(s): Some drugs that target tumor metabolism are minimally effective in vitro but limit proliferation in vivo. Our goal was to develop a preclinical platform enabling us to test drugs that target cancer cell metabolism in combination with radiation therapy in vivo. Here we report the initial results of preclinical studies in autochthonous mouse models of prostate and colorectal cancer using radiation as monotherapy.
Materials/Methods: A dose escalation study was performed in C57BL/6 wild-type mice using 1-5 fraction regimens with a BED of 90, 130, 180, and 250 (a/b = 3). Flank xenograft models of prostate cancer (22Rv1 and PC3 cells) were treated with 24 Gy in 4 fractions or sham irradiated (n = 10 animal per group, 40 animals total). Pb-Cre;Ptenfl/fl;p53fl/fl mice (autochthonous prostate cancer model) age 5 months were randomly assigned to two dose regimens (37.5 Gy in 5 fractions or 45 Gy in 5 fractions) or sham irradiation (n = 11-14 per group). Survival was estimated by Kaplan-Meier Analysis. Colorectal tumors were induced in male and female Villin-CreER;Apcfl/fl mice by intramucosal tamoxifen injection. Mice were assigned 2:1 to 37.5 Gy or sham irradiation (n = 10-15 per group). Treatment effect was measured as percent survival 6 months after tumor induction. In all cases pathologic response rate was determined using serial histologic sections and proliferation by Ki-67 immunohistochemistry.
Results: For a 2 cm field directed at the low abdomen and pelvis of mice, the maximum tolerated dose of radiation was 45 Gy delivered in 5 fractions. The intestinal tract was identified as the dose limiting organ for abdominopelvic radiation. In irradiated flank xenografts, growth delay was observed for all tumors compared with 100% progression in unirradiated controls. Pathologic complete response was observed in 1 of 10 (10%) 22Rv1 xenografts and 4 of 10 (40%) PC3 xenografts. In the autochthonous prostate cancer model, median survival was significantly improved in the 45 Gy (236 days, $P < 0.0001$) and 37.5 Gy (227 days, $P = 0.0007$) arms relative to control (206 days). Irradiated tumors were also smaller relative to control (mean difference -2.637 grams [95% CI -4.617 to -0.6563, $P = 0.0153$); however, all mice eventually died of local tumor progression. In the autochthonous colorectal cancer model, survival at 6 months was significantly improved in the radiation group relative to control (100% vs 40%, $P = 0.0012$). In both prostate and colorectal cancer autochthonous models the proliferation index at time of death (prostate) or 6 months after tumor induction (colorectal) was similar in irradiated and control cohorts.
Conclusion: Hypofractionated radiation therapy can be safely administered to the abdominopelvic region in mice and improves survival in autochthonous mouse models of prostate and colorectal cancer. Radiation can induce pathologic complete responses in flank xenografts, but not autochthonous tumors, suggesting the presence of radioresistant persister cells in these models.

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Proteomic Profiling of Hypoxia-Induced Changes in Cell-Derived Extracellular Matrix From Bladder Cancer Cell Lines

C.Guerrero Quiles,[1] R. Mallender,[1] E. More,[1] J. Humphries,[2] M. Humphries,[2] A. Whetton,[3] A. Choudhury,[4] and C.M.L. West[5]; *[1]Oglesby Cancer Research Centre, University of Manchester, Manchester, United Kingdom, [2]Wellcome Trust Centre for Cell Matrix Research, University of Manchester, Manchester, United Kingdom, [3]Steam and Leukaemia Proteomics Laboratory, University of Manchester, Manchester, United Kingdom, [4]Manchester Cancer Research Centre, Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom, [5]University of Manchester, Manchester, United Kingdom*

Purpose/Objective(s): The extracellular matrix (ECM) is an important component of the tumor microenvironment with key roles in cancer development, metastasis, inflammation and treatment resistance. There are synergies between a cancerous ECM and hypoxia. Hypoxia is a predominant feature of advance bladder cancer. It influences ECM remodeling, and a cancerous ECM can also promote hypoxia signaling through HIF1. We hypothesized that proteomic profiling of the hypoxic-induced ECM will identify potential new biomarkers and therapeutic targets. Our first objective was to identify differentially expressed proteins in the ECM from cells cultured in hypoxia vs normoxia and compare with proteins found in plasma from patients undergoing radiotherapy.
Materials/Methods: UMUC3, J82, RT4 and T24 bladder cancer cell lines were cultured under normoxic (21% O_2) and hypoxic condition (0.2% O_2). Cell cultures were decellularized with NH_4OH, and the cell-derived ECM (CDM) proteins recovered. CDM samples were then in-gel trypsin digested and tandem mass spectrometry performed. Experiments were performed in biological triplicate. Hypoxia-induced cellular and CDM changes were validated by western blotting. In vitro potential biomarkers were validated comparing with LC-SWATH-MS data of longitudinal plasma samples from 10 bladder cancer patients undergoing radiotherapy.
Results: Our results showed a strong influence of hypoxia on bladder CDM. The abundance of 66 out of 186 detected ECM proteins changed significantly ($P < 0.05$) in response to hypoxia in at least one of the cells lines. The results also highlighted diversity of CDM composition in response to hypoxia as only one protein (angiopoetin-4) was up-regulated across all four cell lines. The 66 proteins segregated samples into hypoxic and normoxic groups when analyzed with PCA and heatmap clustering. Gene ontological analysis indicated that the hypoxia-induced changes in CDM proteins have functions related to ECM structure, cell adhesion binding, integrin binding and grow factor activity. LC-SWATH analysis showed that several of the CDM proteins e.g., TGFB1, ANGPTL4, GDF5 were detectable in plasma from bladder cancer patients.
Conclusion: The CDM composition changes in response to hypoxia. The 66 proteins identified stratified bladder cells lines according to oxygenation status and several were identified in plasma samples from bladder cancer patients. This newly identified bladder hypoxic matrisome signature will be evaluated further in plasma samples from a bladder cancer cohort as a potential biomarker for patient stratification to identify those likely to benefit from hypoxia-modifying treatments.

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Radiosensitivity and Immune Cell Infiltration Signature Predict Clinical Outcome of Patients in the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) Study Cohort

B.H. Kang,[1] B.S. Jang,[2] and I.A. Kim[3]; *[1]Department of Radiation Oncology, Seoul National University Hospital, Seoul, Korea, Republic of Korea (South), [2]Department of Radiation Oncology, Seoul National University College of Medicine, Seoul, Korea, Republic of Korea (South), [3]Department of Radiation Oncology, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of Korea (South)*

Purpose/Objective(s): There is growing evidence that tumor radiosensitivity is associated with immune activation in solid tumors. However, previous studies have not examined the relationship between patterns of immune infiltration and radiation response according to diverse molecular subtypes of breast cancer. This study evaluated radiosensitivity and immune infiltration signature to define a group of patients would benefit most from radiation therapy.
Materials/Methods: We performed integrative analyses of the clinical, genomic, transcriptomic, and immunogenomic data to characterize the molecular features associated with radiosensitivity. We analyzed 1,903 data sets of METABRIC Study cohort using the radiosensitivity index (RSI), CIBERSORT and xCell, gene expression deconvolution algorithm which estimates the immune composition of tumor samples. According to