### PI Information
- **Name:** Fornace, Albert M.D.
- **Title:** NSCOR: Space Radiation and Intestinal Tumorigenesis: Risk Assessment and Counter Measure Development
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- **PI Email:** af294@georgetown.edu
- **Fax:** FY
- **PI Organization Type:** UNIVERSITY
- **PI Organization Name:** Georgetown University
- **PI Address 1:** Dept. of Oncology, Lombardi Comprehensive Cancer Center
- **PI Address 2:** Research Building, Room E504, 3970 Reservoir Rd., NW
- **PI Web Page:** http://www9.georgetown.edu/
- **City:** Washington
- **State:** DC
- **Zip Code:** 20007-2126
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- **Contact Monitor:** Simonsen, Lisa
- **Contact Email:** lisa.c.simonsen@nasa.gov
- **Contact Phone:**
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### COI Name (Institution)
- Li, Henghong (Georgetown University)
- Girard, Luc (UT Southwestern Medical Center)
- Ressom, Habtom (Georgetown University)
- Shay, Jerry (UT Southwestern Medical Center)
- Byers, Steve (Georgetown University)
- Datta, Kamal (Georgetown University)
- Batten, Kimberly (UT Southwestern Medical Center)
- Cheema, Amrita (Georgetown University)
- Xie, Yang (UT Southwestern Medical Center)
- Roig, Andres (UT Southwestern Medical Center)
- Richardson, James (UT Southwestern Medical Center)
- Wright, Woodring (UT Southwestern Medical Center)
- Lopa, Mishra (Georgetown University)

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The overall goal of this project is to improve NASA risk estimates for space radiation-induced intestinal tumors. We are employing APC mouse models that have been successfully used to demonstrate radiation-induced intestinal cancer. The APC models have inactivation of one allele of the APC (adenomatous polyposis coli) gene and generally have sporadic loss or truncation mutations of the other gene. This results in mice developing intestinal tumors, called polyps or adenomas and more rarely invasive cancers. Space and terrestrial radiation could differentially perturb other factors of molecular pathways involved in intestinal tumorigenesis and thus initiate increased intestinal tumor development and/or progression.

We continue to employ two models of GI (gastroninestinal) cancers: the APC1638N model, which was generated by a targeted modification of the Apc gene at a position corresponding to aa1638. These mice develop very few adenomas in the small as well as in the large intestine but post-radiation tumor numbers show marked increases. The second model is the CDX2P Apc flox/+ that has one APC allele inactivated almost exclusively in the colon leading to adenomas and subsequent a very low rate of adenocarcinomas in the colon that is increased post-irradiation. Data being collected from these two relevant mouse models will be used to calculate risk estimates for NASA.

The strategy is to quantitatively analyze intestinal tumor incidence and grade following exposure to HZE (high energy) ions, and/or high-energy protons of various energies, LET (linear energy transfer), doses, and dose rates and then to compared to gamma rays in the APC1638N/+ and CDX2P Apc flox/+ models. Based on results in the Shay laboratory, efforts have also included single dose and protracted proton irradiation simulating a solar particle event. We have initiated experiments to assess potential gender and age variation in tumor incidence with space radiation and also to characterize the effects of space radiation in triggering persistent stress responses and their potential effects on tumorigenesis. The plan is also to assess in these mouse models the relative effects of space radiation at other points in the carcinogenic process. Efforts currently and in the future focus on delineating cancer progression-specific biological changes and genomic, proteomic, and metabolomic signatures associated with radiation-induced tumorigenesis, as well as perturbations in tumor suppressor and oncogene-related pathways in radiation-induced tumors.

Since low-LET risk estimates are available for human populations, we will then be able to model the relative risk for space radiation. In particular, this ratio can then be extrapolated to human exposure to low-LET radiation to then make an estimate of risk for colorectal cancer in humans exposed to space radiation compared to the standard risks based on atomic bomb survivors and other exposure populations.

In order to answer the critical question of risk for colorectal tumorigenesis after acute and chronic space radiation exposures, in vitro human cell culture and animal models for CRC initiation and progression are needed. We are investigating the effects of high-LET particle and high-energy protons in colonic epithelial cells with respect to the nature of damage and consequent tumor induction using biological cancer endpoints and systems biology (genomics, proteomics, and metabolomics) approaches. In addition, we propose to compare these results to the biological consequences of low dose rates and mixed fields of radiation to which astronauts are likely to be continuously exposed. The studies of low- and high-LET radiation on colonocytes gain more relevance with increasing age when premalignant adenomas and other colonic lesions such as flat lesions and microadenocarcinomas such as flat lesions become more prevalent. Pre-existing undetectable genetic or chromosomal aberrations in astronauts in addition to de novo mutations resulting from long-term irradiation may increase the chances of colonic stem cell transformation.

There are the following three projects our NSCOR (NASA Specialized Center of Research) has been addressing: 1) Space radiation effects in mouse models of intestinal tumorigenesis; 2) Space radiation effects in neoplastic progression events in normal human colonocytes; 3) Space radiation effects in putative GI stem cells.

Gastrointestinal tumors are frequent in the U.S. and American Cancer Society estimates that there will be 136,830 new cases of colorectal cancer (CRC) with 50,310 persons predicted to die of the disease in 2014. Colorectal cancer when considered in men and women is the second leading cancer killer in the United States (http://www.cdc.gov/).

Considering the high frequency of colorectal tumors in the American population, an even small increase by space radiation could have a major impact on risk estimates and planning of future manned space missions. The effect of complex DNA lesions, which are produced by HZE radiation, on intestinal tumorigenesis may provide insight into mutagenic processes affecting genome integrity and colorectal carcinogenesis.
Protons and high charge and energy (HZE) particles are considered to be major risk factors for humans during space missions. However, the biological effects (such as increases in fatal cancer risk) of protons and HZE particles still need to be more fully characterized. In this study, we used a solar particle event (SPE) simulation at the NASA Space Radiation Laboratory (NSRL) to characterize the effects of low dose rate protons in vivo. Using the colorectal cancer susceptible (CPC;Apc) mouse model, we studied survival and the progression of colon cancer after total body exposure to a simulated solar particle event (SPE) with varying energies (50 – 150 MeV/n) using a total dose of 2 Gy over a 2 hour period (at an average dose rate of 1.67 cGy/min). We also exposed mice to 2 Gy of monoenergetic (50 MeV/n) proton or X-ray (250 kVp, 1 mA, 1.65 mm Al filter) at a dose rate of 20 cGy/min as a reference radiation exposure. The SPE simulation was more effective in inducing an increase in the number of polyps, and the percent of invasive adenocarcinomas compared to monoenergetic acute protons or X-rays. We also observed a chronic/persistent increase in oxidative stress, β-catenin/cyclin D1 signaling and a subset of senescence-associated inflammatory response genes (e.g., Troy, Prox1, and Pla2g2a) as well as a decrease in a subset of circulating miRNAs (e.g., miR-31 and miR-375) that persisted 100 days after exposure to SPE. The mice exposed to 2 Gy of silicon (28Si) particles also show a similar biological effectiveness in increasing colon cancer progression. For comparison, groups of mice were fed with a diet containing the anti-oxidant/anti-inflammatory synthetic tripterpenoid, CDDO-EA, for 3 days prior to SPE or 28Si exposure to determine if mice could be protected from space radiation-induced damage. We found that administration of the CDDO-EA just a few days prior to SPE or 28Si irradiation reduced oxidative stress, chronic inflammatory responses, and cancer progression. These findings suggest that exposure to low dose rate SPE protons and 28Si elicit significant changes in biological effects that have functional consequences on colon cancer progression.

Task Progress on Human Colonic Epithelial Cells (HCECs):

1. To address the cellular function of miR-31-5p, we transfected a miR-31-5p mimic (sense) or inhibitor (antisense) into human colonic epithelial cells and colon cancer cell lines followed by gamma-irradiation. We found that a miR-31-5p mimic sensitized cells to irradiation, while a miR-31-5p inhibitor protected normal colonic epithelial cells against radiation. miR-31-5p regulates mismatch repair (MMR) gene expression, including the human mutL homolog 1 (hMLH1). The miR-31-5p inhibitor failed to modulate radiosensitivity in a hMLH1-deficient HCT116 colon cancer cell line but protected HCT116 3-6 and DLD-1 (both hMLH1-positive) colon cancer cell lines. Our findings demonstrate that miR-31-5p has an important role in radiation responses through regulation of hMLH1 expression. Targeting this pathway could be a promising therapeutic strategy for future personalized anti-cancer radiotherapy.

2. High charge (Z) and energy (E) (HZE) particles in deep space have significantly contributed to the biological effects of space radiation, although they only account for less than 1% of the galactic cosmic rays (GCR) particle fluxes. Previously we have shown that combined radiation exposure of 2-Gy proton (1H) followed by 0.5-Gy iron (56Fe) ion particles increase transformation in human colonic epithelial cells (HCEC CT7). The present study was undertaken to characterize if additional HZE ions such as oxygen (16O) and silicon (28Si) particles also result in increased cell transformation. HCEC CT7 cells irradiated with 1-Gy 16O (250 MeV/nucleon) followed 24 h later by 1-Gy 28Si particle (300 MeV/nucleon) showed an increase in proliferation, anchorage-independent growth, migration, and invasion abilities compared to unirradiated controls. In addition, we found that the β-catenin pathway was activated and that subsets of DNA repair genes were under-expressed in these transformed cells. Pretreatment with the radioprotector, CDDO-Me, 18 h before and during irradiation prevented the HZE-induced transformation. These results can be interpreted to suggest that the mixed radiation exposure of 16O followed by 28Si has carcinogenic potential. Importantly, this transformation can be protected by CDDO-Me pre-treatment.

While we completed a number study objectives described above for the NNX09AU95G and published the data in peer-reviewed journals, our ongoing studies are continuing to qualitatively analyze GI tumor and normal tissue samples collected from the irradiated mice to develop a mechanistic understanding of space radiation-induced enhanced GI tumorigenesis. These studies are continuing as part of our renewed NSCOR on GI carcinogenesis (NNX15AI21G).

ANNUAL PROGRESS REPORTED AUGUST 2014:

Task Progress on Mouse Models of Colon Cancer:

Considering the increased colorectal cancer incidence in A-bomb survivors and the novel characteristics of space radiation, cancer causation by space radiation could potentially be even greater. One general approach for risk assessment is to determine the relative biologic effectiveness (RBE) of various parameters for space radiation compared to terrestrial radiation exposures. For HZE ions there is more uncertainty with RBE for cellular parameters, such as chromosome aberrations and cell lethality, where values obtained from in vitro studies typically ranges from 2 to 11-fold. Previously we have reported the RBE of proton (1.06), 56Fe (1.25), 28Si (1.41), and 12C (0.99) for survival in female C57BL/6J mice. The rationale for this study was that acute toxicity for the planned radiation sources at the proposed energies was not available in mice. However, we do not have sufficient in vivo mechanistic data in human or in animal models representing the human disease to develop reliable risk prediction models for space radiation-induced colorectal cancer (CRC). Considering the limitation of acquiring human data, our study is focused on acquiring sufficient data in mouse models of human CRC with an aim to aid in risk model development for safe human space exploration.

Intestinal tumor incidence was studied in CDX2P Apc flox/+ mice after exposure to acute proton (50 MeV/n), solar particle event (SPE) simulation (50-150 MeV/n), 28Si (600 MeV/n), and 56Fe (600 MeV/n) radiation. The goal was to determine if we observed an increase in the number of polyps/adenomas, adenoma-adenocarcinoma transitions, or the development of metastatic disease after exposure to space radiation. One general approach was exposing these mice to 2 Gy of acute proton (50 MeV/n) or SPE simulation (50-150 MeV/n) significantly increases the incidence of polyps and carcinoma in the CDX2P Apc flox/+ mice. SPE simulation and acute proton increased the incidence of polyps and invasive cancer over the X-ray treated mice. SPE simulation also resulted in an overall decrease in survival compared to unirradiated mice and X-ray treated mice (data not shown). Importantly, overall survival is decreased in wild type mice after exposure to 2 Gy of simulated SPE (mostly at very low energies of 50 MeV/n). We have mice in the colony
already irradiated and will determine in the next 6 months if there are increases in polyp formation or carcinoma with both Fe and Si (single and fractionated doses). We previously conducted a series of experiments with silicon and iron. Even though we used fairly high doses we did show a sparing effect on carcinoma with fractionated (66.7 cGy x 3) Si compared to single doses (2 Gy). We currently have ongoing experiments at lower doses. Interestingly, with Fe as a single dose 1 Gy (600 MeV/n) vs 5 fractioned doses 0.2 Gy x 5, we observe an increase carcinoma with fractionated doses. This is consistent with the previous results using a mouse model of lung cancer progression that we have already completed and published (Delgado et al., 2014: Radiation-enhanced lung cancer progression in a transgenic mouse model of lung cancer is predictive of outcomes in human lung and breast cancer. Clin Cancer Res. 2014 Mar 15;20(6):1610-22). We have gone to lower doses (single and fractionated and these experiments are in progress. We have developed technique to culture stem cells from mouse crypts. Together with our stem-like HCEC cells we now have both mouse and human cells that can be used for space radiation-induced intestinal tumorigenesis experiments.

Intestinal tumorigenesis, quantitatively and qualitatively, are studied in APC1638N/+ mice (6 to 8 weeks old). We are also determining age (6 to 8 wks vs. 20 to 24 wks old mice) and gender (male vs. female) effects of intestinal tumorigenesis. Furthermore, wild type female C57BL/6J mice (6 to 8 wks old) were exposed to 56Fe radiation to understand the long-term perturbation of molecular pathways known to be involved in intestinal tumorigenesis. For intestinal tumorigenesis, APC1638N/+ mice were euthanized 150 days after radiation. For long-term studies, wild type mice were euthanized at 2 or 12 months after radiation exposure. We completed our tumorigenesis data compilation in APC1638N/+ mice after exposure to different doses of 56Fe, 28Si, 12C, and gamma radiation and salient observations are discussed here. Exposure of male and female mice to 0.1, 0.5, and 1.6 Gy 56Fe caused significantly higher intestinal tumorigenesis relative to gamma radiation. We also observed significantly higher tumorigenesis in male and female APC1638N/+ mice after exposure to different doses (0.1, 0.5, and 1.4 Gy) of 28Si radiation relative to gamma radiation. Increased tumor frequency was also observed after different doses (0.1, 0.5, and 2 Gy) of 12C radiation, relative to comparable doses of gamma radiation. Greater tumor frequency was observed in male mice relative to female mice, although the gender difference was less with HZE ions than gamma rays. An important observation is that the intestinal tumorigenesis trend in the current study followed the RBE trend we reported earlier showing highest effects after 28Si followed by 56Fe and 12C relative to gamma radiation. The number of larger tumors (>2 mm size) was higher after exposure to space radiation beams relative to gamma radiation. Similar to intestinal tumorigenesis, colonic tumorigenesis was also significantly increased after different doses of 56Fe radiation in both male and female mice relative to corresponding gamma radiation doses. Significantly increased colonic tumor frequency was observed after exposure to 12C relative to gamma radiation. Additionally, significantly greater colonic tumorigenesis was observed in male and female mice at all the doses of 28Si relative to corresponding doses of gamma radiation. Our study in wild type C57BL/6J mice showed that heavy ion 56Fe radiation increased serum adipogenic hormones and activated IGF1 and leptin signaling pathways in intestine. Activation of IGF1 and leptin signaling, which are known to be involved in maintenance of intestinal homeostasis, have implications for gastrointestinal pathologies including cancer. Overall, heavy ion beams used in the current study induced greater intestinal and colonic tumor frequency and preferentially activated cellular proliferative pathways relative to gamma radiation and this is suggestive of increased gastrointestinal (GI) cancer risk after space radiation exposure.

Task Progress on Human Colonic Epithelial Cells (HCECs):

1. We have developed an isogenic series of HCECs that are sensitized to cancer development.
2. We have conducted a soft agar-based short hairpin RNA (shRNA) screen within colorectal cancer (CRC) candidate driver genes (CAN-genes) using a karyotypically diploid hTERT- and CDK4-immortalized human colonic epithelial cell (HCEC) model and discovered that depletion of 65 of the 151 CAN-genes enhanced anchorage-independent growth in HCECs with ectopic expression of K-RasV12 and/or TP53 knockdown (published).
3. We now constructed an interaction map of the confirmed CAN-genes with CRC non-CAN-genes and screened for functional tumor suppressors. Remarkably, depletion of 15 out of 25 presumed passenger genes that interact with confirmed CAN-genes (60%) promoted soft agar growth in HCECs with TP53 knockdown compared to only 7 out of 55 (12.5%) of presumed passenger genes that do not interact. We have thus demonstrated a pool of driver mutations among the putative CRC passenger/incidental mutations, establishing the importance of employing biological filters, in addition to bioinformatics, to identify driver mutations (this work is now published).
4. We have demonstrated that Nrf2 activation by the synthetic triterpenoids, CDDO-ethyl amide, protect colonic epithelial cells against IR-induced damage in part by enhancing repair of DNA damage. CDDO pre-treatment reduced the frequency of both G1 and S/G2 chromosome aberrations and increased the kinetics of appearance and disappearance of repairosomes (CLIP, Rad51, and 53BP1 foci) formation after IR exposure. CDDO did not protect cells with Nrf2-knockdown. The 53BP1 promoter has three ARE sequences and Nrf2 directly binds to these AREs resulting in increased expression after BARD treatment. In addition, CDDO-EA provided before exposure to a lethal dose of total-body irradiation protected wild type mice from DNA damage and acute gastrointestinal (GI) toxicity and improved overall survival. These results demonstrate that Nrf2 activation by synthetic triterpenoids is a promising candidate target to protect the GI tract against acute IR exposure in vitro and in vivo.

Bibliography Type:

<table>
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<th>Abstracts for Journals and Proceedings</th>
<th>Description: (Last Updated: 02/24/2017)</th>
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<tr>
<td>Suman S, Fornace AJ Jr, Datta K.</td>
<td>&quot;Ionizing radiation decreased Beclin1/LC3B and increased oxidative stress and mTOR via PI3K/Akt to inhibit autophagy and promote intestinal cell proliferation in C57BL/6J mice.&quot; Presented at the 60th Annual Meeting of the Radiation Research Society, Las Vegas, Nevada, September 21-24, 2014.</td>
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</table>
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Cancer Res. 2015 Aug 1;75(15 Suppl):816. [http://dx.doi.org/](http://dx.doi.org/), Aug-2015


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Articles in Peer-reviewed Journals


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