Systems Biology Model of Interactions between Tissue Growth Factors and DNA Damage Pathways: Low Dose Response and Cross-Talk in TGFβ and ATM Signaling

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Abstract:

The etiology of radiation carcinogenesis has been described in terms of aberrant changes that span several levels of biological organization. Growth factors regulate many important cellular and tissue functions including apoptosis, differentiation and proliferation. A variety of genetic and epigenetic changes of growth factors have been shown to contribute to cancer initiation and progression. It is known that cellular and tissue damage to ionizing radiation is in part initiated by the production of reactive oxygen species, which can activate cytokine signaling, and the DNA damage response pathways, most notably the ATM signaling pathway. Recently the transforming growth factor β (TGFβ) pathway has been shown to regulate or directly interact with the ATM pathway in the response to radiation. The relevance of this interaction with the ATM pathway is not known although p53 becomes phosphorylated and DNA damage responses are involved. However, growth factor interactions with DNA damage responses have not been elucidated particularly at low doses and further characterization of their relationship to cancer processes is warranted. Our goal will be to use a systems biology approach to mathematically and experimentally describe the low dose responses and cross-talk between the ATM and TGFβ pathways initiated by low and high LET radiation. We will characterize ATM and TGFβ signaling in epithelial and fibroblast cells using 2D models and ultimately extending to 3D organotypic cell culture models to begin to elucidate possible differences that may occur for different cell types and/or inter-cellular communication. We will investigate the roles of the Smad and Activating transcription factor 2 (ATF2) proteins as the potential major contributors to crosstalk between the TGFβ and ATM pathways, and links to cell cycle control and/or the DNA damage response, and potential differences in their responses at low and high doses. We have developed various experimental approaches to apply to these problems using confocal microscopy and flow cytometry to detail changes at low dose/dose-rate in order to understand individual cell responses, and will establish our mathematical models based on the experimental findings resulting from changes in DNA repair, apoptosis and proliferation.

Relevance Statement:

Establishing the scientific basis of radiation risk as low dose (less than 0.1 Gy) is a primary goal of the DoE Low Dose Research Program. There are large uncertainties in attempts at the

extrapolation of experimental or human epidemiology data from high to low doses. The linear nothreshold model (LNT) remains the chief tool for this extrapolation; however there is continued scientific debate on its validity. Although the LNT model is often justified by DNA damage and mis-repair models of cancer risk, such models are at odds with more recent biological concepts based on the hallmarks of cancer, systems biology approaches, and non-targeted effects. However these more recent scientific concepts have not been developed in a quantitative manner to be used in risk estimation. Our research will support such a goal by developing a mathematical model based on biochemical interactions and experimental systems biology approaches to improve the understanding of the ATM and TGFβ signaling pathways and their cross-talk at low doses. Prediction of possible differences that occur when comparing high versus low doses (<0.1 Gy) of radiation will be investigated. We will utilize a modular system biology approach with long-term applicability as detailed descriptions of the ATM and TGFβ modules can be coupled to other important modules involved in carcinogenesis in the future. Furthermore, we will investigate the role of radiation quality in the ATM and TGFβ pathway responses by characterizing differences between high and low LET radiations.

Summary of Research:

Excellent progress was made with a significant number of publications in high quality research journals including *Science Signaling*, *Radiation Research* and *PLoS One*. Research continues to focus on low dose responses (<0.1 Gy) in human cells and tissue including epithelial cells, and understanding the importance of the TGFbeta-Smad signaling in the DNA damage response, with additional modeling efforts on applications to risk assessment on Earth and in space.

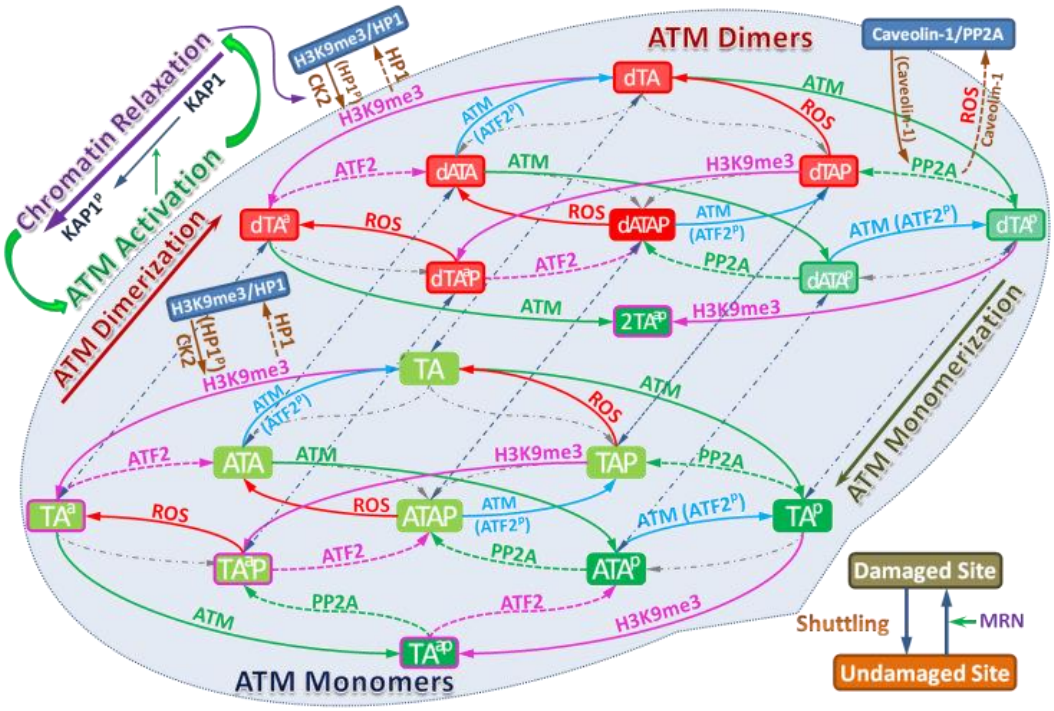
Dr. Eliedonna Cacao was hired as a post-doctoral research under in April of 2015, and as is working on the TGFbeta-Smad models and evaluating dose response models for non-targeted effects for chromosomal aberrations and tumor induction. A summer intern Mr. Samet Ayetekin, an under-graduate student, is working on the ATM modeling studies. A new approach to estimate cancer risks amongst radiation workers for high and low LET radiation exposure was developed based on our more basic studies.

We completed a model of ATM activation and coupled the model with the TGFbeta-Smad signaling pathway and compare to experimental data from our previous DoE Low Dose grant and other data in the scientific literature. We used an innovative modular systems biology model to understand this important signaling pathways in low dose radiation responses (Li et al., 2012; 2016). The model developed (Figure 1 schematic of the model) is the most detailed to date and includes the activation of ATM by both DNA damage and reactive oxygen species (ROS).

Non-targeted effects (NTEs) include bystander effects where cells traversed by heavy ions transmit oncogenic signals to nearby cells, and genomic instability in the progeny of irradiated cells.[15-17] Analysis of the Harderian gland studies (Chang et al., 2016) and experiments for chromosomal aberrations at low dose (Hada et al., 2014; Cacao et al., 2016) suggest a model based on NTEs is favored over a targeted effects (TE) model, where the later assumes a linear

dose response model consistent with DNA damage and misrepair assumptions, while the former suggests a supra-linear dose response occurs which increases risk at low doses. The NTE model is supported by many mechanistic studies using micro-beams to direct radiation to targeted cells, medium transfer from irradiated to unirradiated cells, and inhibitors of reactive oxygen species, gap junctions and signaling pathways. The totality of these studies provide important evidence for NTEs, which should be prioritized in light of the sparsity of both human epidemiology and animal carcinogenesis studies for high LET radiation at the low doses and dose-rates that occur in space. Several additional publications reported on the application of Non-targeted effects to described experiments on chromosomal aberrations and Harderian gland tumors in B6CF1 mice exposed to gamma-rays and heavy ions at low doses. We expect that ATM and TGFbeta-Smad are key regulators of the NTE response dominating low dose radiation effects, and will continues studies along this line of attack in the future.

Figure 1. Biochemical Reactions in the ATM Activation. ATM activation consists in a series biochemical reactions, protein binding/unbinding, including phosphorylation/dephosphorylation, acetylation/deacetylation. In addition, the shuttling of molecules between the DNA damaged site and undamaged site can also be written in terms of reaction.



Journal Articles published as partially of fully supported by Grant:

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2.) Cacao, E., Hada, M., Saganti, P.B., George, K.A., Cucinotta, F.A.: Relative Biological Effectiveness of HZE Particles for Chromosomal Aberrations and Other Surrogate Cancer Risk Endpoints. *PLoS One* 11(4): e0153998, 2016.

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