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Using Genetically Engineered Mice for Radiation Research

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Abstract

The laboratory mouse has been utilized for many decades as a model system for radiation research. Recent advances in genetic engineering now allow scientists to delete genes in specific cell types at different stages of development. The ability to manipulate genes in the mouse with spatial and temporal control opens new opportunities to investigate the role of genes in regulating the response of normal tissues and tumors to radiation. Currently, we are using the Cre-loxP system to delete genes, such as p53, in a cell-type specific manner in mice in order to study mechanisms of acute radiation injury and late effects of radiation. Our results demonstrate that p53 is required in the GI epithelium, to prevent radiation-induced gastrointestinal (GI) syndrome, and in endothelial cells to prevent late effects of radiation. We have also used these genetic tools to generate primary tumors in mice to study tumor response to radiation therapy. These advances in genetic engineering provide a powerful model system to dissect both the mechanisms of normal tissue injury following irradiation and the mechanisms by which radiation cures cancer.

It is a great honor to have been selected to receive the 2010 Michael Fry Research Award from the Radiation Research Society. I would like to begin by thanking the many teachers, from whom I have been blessed to learn, and the many mentors, with whom I have been fortunate to work.

After graduating from Duke University with a degree in Biology, I was an MD--PhD student at Johns Hopkins, where I performed my PhD thesis research in the laboratory of Michael Kastan, MD, PhD. Mike is a pioneer in the field of radiation biology. He identified the signal transduction pathway by which ATM activates the p53 tumor suppressor protein.^{1,2} Mike was an important role model, who showed me how to successfully combine a research career with a focused clinical practice. My introduction to the p53 pathway as a graduate student not only influenced my projects as a post-doctoral fellow, but continues to guide the scientific direction of my own laboratory. At Johns Hopkins, I was also fortunate to collaborate with J. Marie Hardwick, PhD. Marie is a leader in the field of programmed cell death, or apoptosis. She was an active member of my thesis committee and provided sound guidance as I pursued a thesis project studying members of the Bcl-2 protein family during apoptosis.^{3,4,5} This work provided a firm foundation for my post-doctoral projects, where I investigated the role of apoptosis in tumor development and in the acute radiation syndrome.^{6,7}

After graduating from Johns Hopkins School of Medicine in 2000, I completed a year of internal medicine training and then pursued a residency in radiation oncology at Massachusetts General Hospital. I trained with a number of leading radiation oncologists, radiologists, and radiation biologists including William Shipley MD, Chris Willett MD, Tom Delaney MD, Simon Powell MD, PhD, Rakesh Jain PhD, Ralph Weissleder MD, PhD,

Kathy Held PhD, Nancy Tarbell MD, Jay Loeffler MD, Herman Suit MD, DPhil, and Anthony Zietman MD. Simon Powell was a role model for how to be a successful physician-scientist in radiation oncology. He also gave me sage advice to spend whatever time was needed to complete a post-doc before embarking on an independent scientific career. Kathy Held, who served as the 2010 President of the Radiation Research Society, taught the fundamentals of radiation biology to me and the other residents at Harvard. Kathy also generously reviewed my grant applications and provided feedback on my manuscripts. Herman Suit inspired me by combining a tremendous intellect that spanned clinical medicine, radiation biology, and physics with a deep curiosity and an incredible respect for data regardless of whether it confirmed or refuted his own hypothesis. Anthony Zietman taught me numerous pearls of wisdom, and as the residency program director encouraged me to be the first Harvard resident to participate in the Holman Research Pathway and undertake a 21-month research block during my residency training.⁸ When I decided to pursue my post-doctoral training at MIT, Anthony had no hesitation sending me across the Charles River to Cambridge, MA, to work in the laboratory of Tyler Jacks, PhD.

Tyler is a pioneer in generating genetically engineered mice to study cancer. Indeed, he generated some of the first mice with deletions in tumor suppressor genes, including Rb,⁹ NF1,¹⁰ and p53.¹¹ These and other genetically engineered mice generated in the Jacks lab, such as p21^{-/-} mice,¹² Kras^{LA-1} mice,¹³ LSL-Kras^{G12D} mice,¹⁴ and LSL-p53^{R270H} mice,¹⁵ have been widely used by cancer researchers as critical tools to gain key insights into cancer biology and therapy.^{16,17} When I joined the Jacks lab in the fall of 2003, I was very excited to apply these sophisticated genetic tools to study cancer and radiation biology.

Most of these mouse models of cancer utilize the Cre-loxP system. Cre is a site-specific DNA recombinase from the bacteriophage virus that infects *E. coli* bacteria.¹⁸ Cre recognizes short sequences of DNA, termed loxP sites, and acts as a molecular scissors to delete DNA flanked by loxP sites (Figure 1). The Cre-loxP system can be used to “turn on” an oncogene. For example, the Jacks lab utilized genetic engineering to create mice in which Cre specifically turns on the expression of Kras^{G12D}, in which the glycine at codon 12 has been mutated to aspartate. This particular point mutation is frequently observed in a number of human cancers such as pancreatic cancer, non-small cell lung cancer, and colon cancer. These mice contain a transcription/ translation “STOP cassette,” which is flanked by loxP sites, upstream of the mutant Kras allele. Therefore, these mice are called lox-STOP-lox (or LSL) Kras^{G12D}. In the absence of Cre, LSL-Kras^{G12D/+} mice express only the wild-type Kras allele. In the presence of Cre, the STOP cassette is excised. Therefore, oncogenic Kras^{G12D} is expressed from the endogenous Kras promoter at physiological levels. In addition, the Jacks lab generated LSL-p53 mice, in which a STOP cassette was inserted upstream of the p53 tumor suppressor gene.^{15,19} Because p53 is one of the most commonly mutated tumor suppressor genes in human cancer, the Jacks lab used LSL-p53^{R270H} point mutant mice to generate a variety of mouse models to study cancer, including non-small cell lung cancer²⁰ and pancreatic cancer.²¹ Working with Andrea Ventura, another talented post-doc in the Jacks lab, I utilized the LSL-p53 wild type mice (termed p53^{LSL}) to study the role of p53 in tumor maintenance.

Mice lacking p53 are tumor prone.^{11,22} However, it was unclear whether loss of p53 function was only required for tumor initiation, or if loss of p53 function was also required for tumor maintenance. To study the role of p53 in tumor maintenance, we crossed the p53^{LSL} mice with Cre-ER^{T2} mice.¹⁹ Using the ubiquitous ROSA26 promoter, these mice express Cre fused to the modified estrogen receptor ER^{T2}. The ER^{T2} sequesters Cre in the cytoplasm until it binds 4-hydroxy-tamoxifen. Therefore, in p53^{LSL/LSL}; Cre-ER^{T2} mice, Cre is not able to recombine loxP sites in the absence of tamoxifen. However, in the presence of tamoxifen, Cre can enter the nucleus, delete the STOP cassette, and restore wild-

type p53 expression from its endogenous promoter. We generated cohorts of p53^{LSL/LSL}; Cre-ER^{T2} mice, which developed spontaneous lymphomas and sarcomas that were detected with MRI. When these mice were treated with tamoxifen to restore p53, we observed tumor regression.¹⁹ At the same time, other laboratories reported similar results using different mouse models. Scott Lowe's lab utilized *in vivo* shRNA to knock down p53 in a mouse model of liver cancer and demonstrated that loss of p53 function was required for tumor maintenance in a model of hepatocellular carcinoma.²³ In addition, Gerard Evan's lab utilized p53-ER mice in which p53 is sequestered in the cytoplasm. Therefore, these mice develop thymic lymphomas. When the Evan's lab treated these mice with tamoxifen to turn on p53, they also observed regression of the thymic lymphomas.²⁴ More recently, Wang and colleagues compared the effect of restoring wild-type p53 (p53^{LSL} mice) in tumors with no p53 or R172H mutant p53.²⁵ They confirmed that restoration of wild-type p53 in p53 null tumors caused tumor regression. Interestingly, they observed that restoring p53 in tumors that also express R172H mutant p53 did not cause tumor regression, but instead halted tumor growth. Taken together, these results collectively demonstrate that loss of p53 function is required not only for tumor initiation, but also for tumor maintenance.

As a post-doc, I also utilized the Cre-loxP system to study the acute radiation syndrome. Following whole body irradiation (WBI), the bone marrow is depleted and leads to a lethal hematopoietic syndrome within 30 days.²⁶ A higher dose of WBI causes a mixed hematopoietic/gastrointestinal (GI) syndrome with lethality within 10 days.²⁶ Kathy Mason and co-workers²⁷ and Terry and Travis²⁸ had previously shown that survival from this mixed hematopoietic/GI syndrome can be rescued with a bone marrow transplant. However, these groups also found that a bone marrow transplant no longer improved survival once the WBI dose exceeded the threshold that caused lethality by whole abdominal irradiation. Remarkably, bone marrow transplant also failed to rescue mice that received lethal doses of whole abdominal irradiation. These results demonstrated that a dose window exists which allows rescue with bone marrow transplant for WBI lethality caused by a mixed hematopoietic/GI syndrome, but at higher doses of WBI or with whole abdominal irradiation, lethality is a result of the GI syndrome. Therefore, for our studies of the acute radiation syndrome, we utilized WBI to study the hematopoietic syndrome and sub-total body irradiation (SBI) to study the GI syndrome.⁷

To test the hypothesis that radiation-induced apoptosis controls the acute radiation syndrome, I utilized mice lacking the pro-death Bcl-2 family proteins Bak and Bax.²⁹ At least one of these proteins must be expressed for a cell to die by the intrinsic pathway of apoptosis.³⁰ Because mice lacking Bak and Bax often die during development, we utilized transgenic mice that specifically express Cre in different cell types. For example, we utilized Tie2Cre mice to delete Bax specifically within hematopoietic cells and endothelial cells.⁷ When Bax was deleted in Tie2+ cells in Bak^{-/-} mice (i.e., Tie2Cre; Bak^{-/-}; Bax^{FL/-}), they were protected from the hematopoietic syndrome following WBI. However, neither single Bak^{-/-} or Bax^{-/-} mice nor the compound mutant Tie2Cre; Bak^{-/-}; Bax^{FL/-} mice were protected from SBI, indicating that the intrinsic pathway of apoptosis in Tie2+ cells does not regulate the GI syndrome after SBI.⁷ We also utilized VillinCre mice to delete Bax specifically in GI epithelial cells in Bak^{-/-} mice (i.e., VillinCre; Bak^{-/-}; Bax^{FL/-}). However, these mice were also not protected from SBI, indicating that the intrinsic pathway of apoptosis in the GI epithelial cells does not regulate the GI syndrome.⁷

These results were surprising, as I had anticipated that apoptosis would regulate both the hematopoietic syndrome and the GI syndrome. Instead, our results indicated that the hematopoietic syndrome is regulated by the intrinsic pathway of apoptosis, but the GI syndrome is regulated by a cell death pathway that is independent of the intrinsic pathway of apoptosis. In the literature, there was conflicting data regarding the role of p53 in regulating

the GI syndrome. Although some investigators had reported that deletion of p53 did not affect the GI syndrome,³¹ others found that mice lacking p53 are sensitized to the GI syndrome.³² As p53^{-/-} mice are resistant to radiation-induced apoptosis of the GI epithelium,³³ we next utilized the Cre-loxP system to investigate the role of p53 in the GI syndrome. We deleted p53 in the GI epithelium with VillinCre and observed that VillinCre; p53^{FL/-} mice were sensitized to the GI syndrome.⁷ Although the acute radiation syndrome was not affected in Tie2Cre; p53^{FL/-} mice (where p53 is deleted in endothelial and hematopoietic cells), these mice all became moribund from late effects of radiation.⁷ These results demonstrated that p53 acts within the GI epithelium to protect mice from the acute GI syndrome independent of the intrinsic pathway of apoptosis. Moreover, these results indicate that p53 also functions in endothelial and/or hematopoietic cells to prevent late effects of radiation. Recently, Leibowitz and colleagues also reported that mice lacking p53 are sensitized to the GI syndrome because they undergo aberrant cell cycle progression and non-apoptotic death.³⁴

After completing my residency, I joined the faculty of Harvard Medical School and cared for patients on the sarcoma service at Massachusetts General Hospital. I enjoyed working with an extraordinary multi-disciplinary team that included Francis Hornicek MD, PhD and Tom Delaney MD. Having defined sarcoma as my clinical area of focus, I continued my post-doc in the Jacks lab and used the Cre-loxP system to develop a mouse model of soft tissue sarcoma.⁶ To develop this model, I utilized mice with conditional mutations in Kras and p53. I activated these mutations by injecting an adenovirus expressing Cre (Adeno-Cre) into the muscle. Remarkably, two to three months after injection, soft tissue sarcomas developed at the site of injection (Figure 2A) that not only resembled human sarcomas by histology, but also by their predilection to metastasize to the lungs.⁶ This primary mouse model of soft tissue sarcoma is spatially- and temporally-restricted. Because the tumors develop at a defined location, it is useful for studies of surgery⁶ and radiation biology.³⁵ Indeed, working with a colleague in surgical oncology, Sam Yoon, I utilized this mouse model of soft tissue sarcoma to demonstrate improved growth delay by the combination of radiation therapy with the tyrosine kinase inhibitor sunitinib.³⁵ The same mice with conditional mutations in Kras and p53 can be utilized to generate primary non-small cell lung cancer by delivering Adeno-Cre via intranasal inhalation.²⁰ We have used micro-CT to image these lung tumors (Figure 2B) and measure the tumor response to radiation therapy.³⁶ I anticipate that these kinds of genetically engineered mouse models of cancer will be useful to dissect the mechanisms of tumor response to radiation therapy.

In 2007, I returned to my alma mater, Duke University, to care for patients within the multi-disciplinary sarcoma center and to start my own laboratory. I am grateful to my Department Chair, Chris Willett MD, for giving me the time and resources to build a successful independent laboratory. I have been very fortunate to work with an incredible group of graduate students, post-doctoral fellows, and technicians. We are using genetically engineered mice to study sarcoma development and metastasis,³⁷ tumor response to radiation therapy, and mechanisms of acute injury and late effects from radiation.

I would once again like to thank the Radiation Research Society for this honor. I would like to close by quoting Dr. Fry from his paper entitled "Experimental Carcinogenesis: What have we learned?"³⁸:

Animal experiments on radiation carcinogenesis have provided information at a frustratingly slow rate, but they have provided insights important for the understanding of carcinogenesis in general that cannot be obtained as yet in other ways. Animal experiments continue to be vital for (1) the study of mechanisms, (2) the establishment of generalizations, (3) the elucidation of dose-response and time-

dose relationships, and (4) the determination of dose distributions and their consequences, particularly in the use of radionuclides.

I believe that this statement can be extended to genetically engineered mice used for radiation research. While work with genetically engineered mice can be frustratingly slow, for many critical questions in radiation biology, genetically engineered mice are a powerful model system to dissect mechanisms of normal tissue injury following irradiation and to understand how radiation cures cancer.

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Figure 1.

Schematic of the Cre-loxP system. DNA with exons 1 and 2 are depicted as rectangles. Exon 1 is flanked by loxP DNA sequences depicted as triangles. In the absence of Cre recombinase, the DNA is intact. In the presence of Cre recombinase, which is represented by the scissors, recombination occurs at the loxP sites, which deletes the DNA (including exon 1) that is flanked by loxP sites.

Figure 2. Genetically engineered mouse models of cancer. A) Mice with conditional mutations in Kras and p53 (i.e. LSL-Kras^{G12D}; p53 FL/FL mice) were injected into the muscle with an adenovirus that expresses Cre recombinase. A primary soft tissue sarcoma developed at the site of injection. B) Mice with conditional mutations in K-ras (i.e. LSL-Kras^{G12D} mice) inhaled an adenovirus expressing Cre recombinase, and a micro-CT was performed after 5 months to detect primary non-small cell lung cancers.