Mouse Models for Radiation-Induced Breast Cancer

Leena Rivina1, Michael Davoren1,*, Robert H. Schiestl2

1Department of Environmental Health Sciences / 650 Charles E. Young Dr. South, University of California, CHS 71-295, Los Angeles, CA 90095, USA
2Department of Environmental Health Sciences, Department of Pathology and Laboratory Medicine; Department of Radiation Oncology; JCCC Healthy and At-Risk Populations Program Area 650 Charles E. Young Dr. South, University of California, CHS 71-295, Los Angeles, CA 90095, USA
*Corresponding Author: mdavoren@ucla.edu

Abstract Radiation is a widely known, and prototypical, inducer of genotoxic damage. With every moment of exposure to ionizing radiation, lesions and breaks are induced in the DNA, increasing an individual’s lifetime risk of developing cancer. At the same time, radiation therapy is a key part of the effective treatment of the very same disease. Radiation therapy is effective, capable of shrinking and even eliminating tumors. In conjunction with surgery, its use is extremely common for the treatment of breast cancer. Even when radiation is our ally, however, the risks remain. Therapeutic use to treat existing cancers paradoxically leads to the incidence of secondary, radiation-induced neoplasias. One strategy to reduce this secondary risk while still encouraging the use of radiotherapy to its full potential would be the development co-administered therapeutic compounds or strategies designed to preferentially protect healthy cells while leaving cancer cells vulnerable. The development and efficacy testing such agents would require not only extensive in vitro testing, but also a well investigated set of in vivo models to actively recapitulate the complex nature of radiation-induced carcinogenesis. The laboratory mouse Mus musculus is probably the best choice for this endeavor. As a cancer model it possesses a combination of favorable attributes: a well annotated genome, molecular and physiological similarities with man and other mammals, and a small size and high breeding rate for ease of use. This work will focus on the description of m. musculus inbred and F1 hybrid animal models of radiation-induced breast cancers and their associated molecular pathologies.

Keywords Radiation Carcinogenesis, Breast Cancer, Animal Models, Secondary Cancers

1. Introduction

As the “Greying” of the USA and other western nations continues, diseases of the aged will demand a greater and greater proportion of our attention. It is currently predicted that roughly half of all U.S. citizens will receive a cancer diagnosis at some point in their life, and of that of these another half will take a course of treatment including radiation therapy [1, 2]. Radiation has a number of uses as a therapeutic agent, and can be administered alone or with concurrent treatments such as chemotherapy or immunotherapy. In the case of breast cancer in particular, radiation therapy is commonly employed post-surgery to ensure total removal of cancerous cells, and increases the survival rate [3]. Beyond the direct irradiation of tumors, radiotherapy is also used to initiate immunosuppression for bone marrow, stem cell and organ transplantation [4]. Radiation’s useful killing power comes at a cost, however, as it is not specific for cancer cells. Any dividing cell is particularly vulnerable to the genotoxic effects of radiation, and the collateral effects can result in a variety of acute toxicities and secondary chronic problems. The irony of this lies in radiation’s propensity to cause DNA damage that might one day lead to cancer itself; a beam of radiation that obliterates an existing cancer could simultaneously sow the seeds of a secondary radiation-induced cancer that will rear up later in the patient’s life. [5, 6].

The first clear avenue towards reducing these risks lies in improving the radiotherapy equipment itself, as a precise, targeted beam minimizes collateral damage to healthy tissue while pumping the greatest proportion of its energy into the targeted tumor. Indeed, great advances have already been made in this regard. Accurate delivery of radiation increases the patient’s maximum tolerated dose and increases the therapeutic ratio [7, 8]. Tumor growth, however, is a complex and insidious thing. The complicated nature of tumor interactions with healthy tissue and a degree of uncertainty about where potentially metastatic cells could remain makes avoiding all collateral exposure unfeasible during most clinical irradiations. This leads to practical limits as to how much radiation can be employed without overly damaging the rest of the patient. For example, even with modern 3D conformal radiation therapy, breast cancer irradiations still carry risks, including the weakening of the
patients’ ribs [9]. Therefore, technological solutions represent only one piece of the puzzle. If biological, or chemical, interventions against radiation damage could be employed simultaneously in such a way as to maximize the protection they afford healthy tissues, and minimize the protection to target cancer tissues, they would allow for radiotherapy to become much more effective. Such a treatment could probably exploit subtle differences in the metabolism or genome of healthy and cancerous cells that the raw power of radiation cannot distinguish.

Chemical or biological agents capable of this feat can be divided into three classes based relative time of effective application. The first class, radiation protectors, consists of agents applied prior to radiotherapy itself. The second class, radiation mitigators, would be given post-exposure (PE) of radiation, but prior to the onset of radiation-induced symptoms. The third class of therapies would be administered after the onset of radiation-induced symptoms [10]. There is currently only one agent FDA approved for this type of treatment. This drug, Amifostine, is a member of the first category, usually injected intravenously a few minutes prior to radiotherapy [11]. Unfortunately, it has a number of drawbacks including cytotoxic side effects and limits to its effectiveness. A wider variety of pharmaceuticals would be extremely valuable. In recognition of this need, the National Cancer Institute (NCI) and National Institute of Allergy and Infectious Diseases (NIAID) has proposed an algorithm to select of agents of the classes previously discussed for preclinical and clinical development [12]. The validation of candidate agents in animal models is an important criterion of these algorithms. For this reason, a comprehensive description of available animal models that accurately reproduce the human phenomena of radiation-induced secondary cancers will be extremely valuable to researchers developing an experimental plan to test such agents. Reviews by other groups already describe models focused on the more acute toxicities associated with radiation exposure [13]. The purpose of this work is to provide an updated review of select inbred mouse models that may be used in preclinical settings in order to test the efficacy of agents specifically intended to protect, mitigate or treat radiation-induced breast carcinogenesis.

2. Methods

2.1. Research Strategy

The laboratory mouse, Mus musculus, has long been one of the premier species for in vivo research. The fact that, as a mammal, it can mimic human disease and physiology so closely has always been its greatest strength. Its small size and short generation time also contribute to its ease of use in a wide variety of experiments. Over time, the scientific community has been able to replace many live animal models with simpler, cell culture based systems, for focused lines of inquiry. However, these systems still have trouble accurately recapitulating extremely complex systems, like the study carcinogenesis and its corresponding pathologies. At this time, there is no replacement for the venerable mouse when all body systems must be simultaneously considered in a model. This is not to say that murine experimental systems have not continued to dynamically develop, though. On the contrary, the laboratory mouse has undergone a significant evolution in its complexity over the course of the last century. Researcher continue to examine the details of the mouse genome and develop precise techniques to manipulate it. Through this process, we continue to update mouse models, mimicking progressively more precise aspects of human cancer. Through the use of genetically engineered mice (GEM), modern researchers can use models expressing tumors inducible by specific carcinogen, mice capable of hosting human cancer cells, and even humanized mice that express human genes. These modified strains accurately represent the underlying pathophysiological and molecular features of human cancers, and for reason have replaced inbred, genetically homogenous strains that have historically been used in most environmental cancer induction studies [14]. Compared to GEM and their programmed “cancer development roadmaps”, the development of tumors in inbred strains in much less predictable. Although GEM are excellent choice for certain studies, the fact that they are characteristically designed to follow an exact path of carcinogenesis progression makes it difficult to use them to study alternative mechanisms of induction. Other times, the fact that they must be so specifically designed often means that a GEM model of a particular carcinogenesis question is overly rare or expensive. The use of inbred strains therefore remains an absolutely critical part of cancer research. As more “general” models of carcinogenesis, inbred mice have led to results important in the very development of GEM, including the discoveries of oncogenes, tumor suppressors, experimental compounds [15].

This review focuses on mouse models of radiation-induced secondary cancer of the breast, one of the most common secondary cancers that arise post radiation therapy [6]. The description of these models is intended to promote their use in the development of interventions and compounds designed specifically to either treat these malignancies or reduce the risk of their formation.

2.2. Inclusion Criteria

This review is specifically focused on murine models of breast cancer designed to induce following exposures to low-LET gamma- and X-ray radiations, using both high total dose and high dose-rate. Carcinogenesis induced from high-LET radiation, genetically engineered mouse models, and xenograft models are outside of the scope of this work. In this work, only inbred mice with cancer inducible by a single total body irradiation (TBI) are described. The model strains discussed tightly mimic the underlying molecular pathologies of each type of cancer as observed in humans, maximizing their clinical relevance.
3. Results and Discussion

3.1. Radiation-Induced Breast Cancer

Japanese female survivors of the atomic bomb attacks, females subjected to diagnostic fluoroscopes in Massachusetts tuberculosis sanatoria, and women treated for postpartum mastitis in New York form three core groups providing compelling epidemiological data linking radiation exposure and breast cancer [6]. Data from the Japanese atomic bomb cohort in particular, demonstrates that breast carcinoma (BC) risk increases by a greater extent than all other solid tumor risks upon exposure to IR [5]. In the Massachusetts study, females exposed to over a hundred separate instances of diagnostic x-rays were shown to be 80% more likely to develop breast tumors [16]. Newer reports continue to emerge implicating radiation therapy as a causative agent in secondary breast cancers, and demonstrate dependency on age of exposure. Up to 35% of women treated for Hodgkin’s disease with radiation therapy at an early age developed breast cancer by the age of forty. The studies of Bhatia and Sankila give an approximate IR-induced BC latency period of 10 years following radiation exposure [17, 18]. Stovall and colleagues have reported that an absorbed radiotherapy dose of over 1Gy to the contralateral breast is linked to a high risk of secondary de novo contralateral breast cancer (CBC) [19]. Reproductive history is also a factor in CBC risk. Women who did not have a child prior to their first diagnosis of cancer were more likely to develop CBC after radiotherapy than age-matched controls [20].

Ionizing radiation is a well-established etiological agent in both murine and human breast cancer [21-28]. Mammary cancer mouse models are invaluable to the study of both murine and human breast cancer biology when DeOme and colleagues introduced a murine orthograft breast cancer model. The model consists of clearing the mammary fat pad from a 3-week-old female virgin mouse, followed by a transplant of a 1mm duct fragment from a donor mouse with hyperplastic lesions [33, 34]. Ethier and Ullrich successfully adopted this model from the original strain into BALB/c mice, using it extensively to demonstrate differences in sensitivity between strains and associated molecular mechanisms [32, 35-37]. Additionally, Dr. Barcellos-Hoff and colleagues employed this model, further revolutionizing the cancer research field by demonstrating the importance of tissue microenvironment in the breast carcinogenesis process [38-41].

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Mouse Strain</th>
<th>Age</th>
<th>Sex</th>
<th>Dosage</th>
<th>Fractionation</th>
<th>Latency</th>
<th>Spontaneous Frequency</th>
<th>Induced Frequency</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>BALB/c</td>
<td>12 weeks</td>
<td>Female</td>
<td>2.0 Gy TBI</td>
<td>Single</td>
<td>~24 months</td>
<td>8%</td>
<td>22%</td>
<td>Ullrich, 1983</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>BALB/c orthografi</td>
<td>12 weeks</td>
<td>Female</td>
<td>1.0Gy TBI of donor cells</td>
<td>Single</td>
<td>10 weeks</td>
<td>&lt;1%</td>
<td>Dysplasia ~75%</td>
<td>Tumors ~25% (dependent upon donor cell passage)</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>BALB/c chimera</td>
<td>12 weeks</td>
<td>Female</td>
<td>4.0Gy TBI of host</td>
<td>Single</td>
<td>6 weeks</td>
<td>~19%</td>
<td>~81%</td>
<td>Nguyen et al. 2011</td>
</tr>
</tbody>
</table>

Table 1. Induction of Breast Cancer in Mice with Low-LET Ionizing Radiation

3.1.1. BALB/c Whole-Body Exposure Model

Original studies in the BALB/c female whole-body irradiation model have shown an increase in mammary carcinogenesis, from a background frequency of around 8% to about 22% within the mouse’s lifetime. The mammary adenocarcinoma induction method consists of irradiating 12 week old females with a total dose of 2.0 Gy, at the relatively high dose-rate ~ 0.35Gy/min; irradiation with the same total dose at a much smaller dose-rate of 0.083Gy/day resulted in roughly half the tumorigenesis frequency, only ~13% [28]. The high dose rate seems to be key; even a dose of 0.25Gy at 0.35Gy/min induces mammary tumors in about 20% of mice [30]. Irradiation increases the incidence of breast adenocarcinomas, but does not seem to affect latency relative to spontaneously arising tumors. Hyperplastic lesions in the ductal dysplasia are detected 12-14 months after IR exposure, prior to appearance of the tumor proper [31]. Radiation-induced breast adenocarcinoma sensitivity in the BALB/c female has been attributed to polymorphisms of Prkdc, a DNA-dependent protein kinase, involved in DNA repair and post-IR cell signaling [32]. An unfortunate possible downside of this model, however, is its high rate of concurrent ovarian tumor development, detected in over 90% of autopsied mice [28].

3.1.2. BALB/c Syngenic Transplant Model

In 1959, a great advance was made in the field of breast cancer biology when DeOme and colleagues introduced a murine orthograft breast cancer model. The model consists of clearing the mammary fat pad from a 3-week-old female virgin mouse, followed by a transplant of a 1mm duct fragment from a donor mouse with hyperplastic lesions [33, 34]. Ethier and Ullrich successfully adopted this model from the original strain into BALB/c mice, using it extensively to demonstrate differences in sensitivity between strains and associated molecular mechanisms [32, 35-37]. Additionally, Dr. Barcellos-Hoff and colleagues employed this model, further revolutionizing the cancer research field by demonstrating the importance of tissue microenvironment in the breast carcinogenesis process [38-41].
Ethier and Ullrich also employed the “cell dissociation assay,” an in vitro/in vivo model in which 12 week old virgin donor BALB/c females are whole body irradiated with a total dose of 1.0 Gy, with mammary tissues removed at 24 hours post-exposure. A single-cell suspension of 104 cells from these donor animals is then injected into 3 week old virgin BALB/c females with cleared mammary fat pads. 10 weeks after the procedure, recipient mice are sacrificed and the outgrowths removed and analyzed for ductal architecture pathologies. Normal outgrowths contain 2 to 3 terminal ducts, are capped by end buds in the fat pad, and resemble anatomically correct ducts. Abnormal outgrowths, on the other hand, have up to 10 or more terminal ducts capped with hyperplastic end buds. These abnormal architectures are assigned an arbitrary classification between I and III, with Class III designated as the most severe [36, 42, 43].

In another series of elegant experiments, Ullrich and colleagues demonstrated that cells harvested from an irradiated donor, passaged in vitro, and finally transplanted into unirradiated recipient mice develop into either dysplasia or adenocarcinomas. The result depended upon time of harvesting and number of passages in culture prior to implantation. Cells harvested 52 weeks post-IR and injected into recipient host tended to regenerate dysplastic outgrowths at a high rate (3 in 4) and develop into tumors (1 in 4). Cells harvested at 1-16 weeks developed into normal outgrowths unless they underwent extensive in vitro passaging. The dysplasia and tumors observed resembled in situ tumorigenesis, with leukocyte infiltrations and angiogenesis [31].

Barcellos-Hoff and Ravi established a chimeric radiation model of their own [44] in which the fat pads of a BALB/c mouse host are cleared at 3-weeks of age, with the same mice whole body irradiated with 4.0 Gy at 10-12-weeks of age. Three days later these hosts receive a transplant of immortalized but non-malignant COMMA-D mouse epithelial cells derived from midpregnant BALB/c females [45]. 6 weeks post-IR the cells injected into irradiated host had 81% tumor penetrance, compared to only 19% of cells injected into an unirradiated host. This syngeneic model demonstrates that radiation causes changes in the stromal microenvironment which contribute to carcinogenicity [44]. Tissue, rather than cell suspension based alternative model can achieve a similar result, with a 1mm3 formed duct epithelial fragment acquired from a wildtype donor or a donor primed for neoplastic development transplanted into the irradiated host whose mammary fat pads have been cleared [46].

3.1.3. Breast Cancer-Associated Molecular Pathologies

Cell lines derived from female BALB/c mice and harvested at 4 weeks (EF42) or 16 weeks (EF137) after 1 Gy whole body irradiation have been used for some time to examine molecular pathologies leading to tumorigenesis in vitro or transplanted into recipient mice for in vivo studies. Cell culture studies point to a number of familiar players in the oncogenic protein scene. Reduced or absent Rb can be detected in EF42 after 11 passages in EF42 cells, and after only 6 passages in the EF137 line. Mutant p53 is present in 95% of these cells after 20 passages and can even be detected as early as passage 6 in 1-5% of cultures, suggesting p53 mutation is an early transformation event in these preneoplastic cells. Angiogenesis is usually detected after about 20 passages [31]. In in vivo transplantation studies, Ethier and Ullrich reported that introducing ten times the amount of cells at ~105 actually decreased both the frequency and severity of observed dysplasia, compared to an injection of 104 cells [36, 42]. This suggests that replicative stress may be contributing to faster and more prominent progression into ductal dysplasia.

Barcellos-Hoff and colleagues have linked the rapid remodeling of the irradiated mammary gland microenvironment to changes in both the extracellular matrix and latent TGF-β expression [38, 47-49], later showing that this accelerates tumor progression [46]. Transforming growth factor beta, TGF-β, is involved in the regulation of a variety of cell processes, including cell cycle control, apoptosis, and cell differentiation [48, 50]. Activation of TGF-β as a result of radiation has been implicated to influence cell fate decisions and DNA-repair kinetics in an ATM-dependent manner [51, 52].

Radiation chimera models are able to capture prominent features of breast cancers thought to arise following irradiation, even though the transplanted epithelium itself has not been irradiated. IR-associated human breast cancer arises from the duct cells and often infiltrates the rest of the breast tissue [6], a progression similar to observed in transplantation models. Functionally p53 negative tumors induced in transplanted epithelium were estrogen receptor (ER) negative [46], akin to that observed breast cancer of previously irradiated women [53]. The Rb deficiencies observed by Ullrich and Preston in neoplastic duct cells are also often reported in human breast cancer correlating with a highly invasive tumor phenotype [54].

4. Conclusions

Mice provide a particularly useful model for the study of any cancer’s progression, and breast cancer is no exception. The combination of their small size and rapid rate of reproduction essentially makes for a tiny model of a living human body, allowing for the interaction of numerous biological systems that would be nearly impossible to fully consider in vitro. Of course, humans and mice still have their own quirks of genetics and physiology which must always be considered before making a conclusion based on a mouse model. Thus, when considering the ideal mouse model of any particular cancer, we must prioritize features based on two main goals: similarity to the corresponding human disease, and experimental practicality. The first goal is probably the most important for obvious reasons of relevancy; an ideal model for this criterion will produce tumors nearly identical to the corresponding human cancer in
onset, progression and underlying pathology. When developing a model for laboratory use, though, it is also important to have a “focused” model. This would be a model in which one particular cancer can be examined, to the greatest statistical significance, with the least number of animals. For these reasons, our ideal RI-cancer model mouse will also possess a low spontaneous background frequency of the desired malignancy, a short latency period after induction, and avoid co-developing cancers at alternative sites. Invariably, researchers must choose to compromise on some of these features, choosing a model that has the best combination of characteristics for a given study. When features must be sacrificed, it is generally easier to compromise on those contained under the “experimental practicality” umbrella, as these can be compensated for by increasing the number of subjects studied and careful experimental design. Researchers should take great pains to avoid compromising on the accurate molecular and pathophysiological emulation of human RI-cancers, as simulating these to the greatest possible extent is the entire point of in vivo research. The mouse models presented in this review each contain their own combinations on compromise on these features, but all demonstrate strong molecular and phenotypic correlations to salient features of the human cancers they are meant to represent. It is up to the researcher to pick the model best for their specific study.

There are a number of important questions which currently need to be addressed in radiation-induced secondary cancer research, and animal models will be crucial to understanding them. In humans, radiation-induced secondary cancers are still often difficult to discern from primary tumors. Elucidation of differences in their respective molecular signatures will go a long way towards more successfully differentiating between the two in a clinical setting, which would hopefully lead to more targeted treatments. Another important area which will rely on in vivo testing is the burgeoning field of radiation mitigators. Radiation therapy’s raw power against cancer cells is tempered by the risk of inducing these secondary cancers, as well as the sheer collateral tissue damage that is unavoidably inflicted on a cancer patient. If a compound could be introduced before, during, or after radiotherapy to differentially protect healthy cells, the possibilities would be enormous. Current radiation therapy technology could have its power drastically increased without a guaranteed concurrent increase in collateral damage. Currently, only Amifostine is FDA-approved for use in this way [11]. Its use, however, is limited by its cytotoxicity. The discovery of new compounds with increased efficacy and less side effects is a particularly attractive field. The mouse models discussed in this paper provide a relatively accurate recapitulation of the complicated, interwoven body systems which would be involved in the testing of such drugs, and are likely to be a cornerstone of any productive research on the topic.

Acknowledgments

The work has been supported by grant No. 1 U19 A1 67769-01 from the National Institute of Allergy and Infectious Disease. The authors also wish to thank the NIEHS Training Grant in Molecular Toxicology for funding provided to Michael Davoren.

REFERENCES


[38] Barcellos-Hoff MH: Radiation-induced transforming growth factor beta and subsequent extracellular matrix reorganization


