Radiation-Induced Mammary Carcinogenesis in Rodent Models: What’s Different from Chemical Carcinogenesis?

Tatsuhiko IMAOKA*¹, Mayumi NISHIMURA¹, Daisuke IIZUKA¹,², Kazuhiro DAINO¹, Takashi TAKABATAKE¹, Micko OKAMOTO¹, Shizuko KAKINUMA¹ and Yoshiya SHIMADA*¹

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Ionizing radiation is one of a few well-characterized etiologic factors of human breast cancer. Laboratory rodents serve as useful experimental models for investigating dose responses and mechanisms of cancer development. Using these models, a lot of information has been accumulated about mammary gland cancer, which can be induced by both chemical carcinogens and radiation. In this review, we first list some experimental rodent models of breast cancer induction. We then focus on several topics that are important in understanding the mechanisms and risk modification of breast cancer development, and compare radiation and chemical carcinogenesis models. We will focus on the pathology and natural history of cancer development in these models, genetic changes observed in induced cancers, indirect effects of carcinogens, and finally risk modification by reproductive factors and age at exposure to the carcinogens. In addition, we summarize the knowledge available on mammary stem/progenitor cells as a potential target of carcinogens. Comparison of chemical and radiation carcinogenesis models on these topics indicates certain similarities, but it also indicates clear differences in several important aspects, such as genetic alterations of induced cancers and modification of susceptibility by age and reproductive factors. Identification of the target cell type and relevant translational research for human risk management may be among the important issues that are addressed by radiation carcinogenesis models.

INTRODUCTION

Ionizing radiation is one of the few well-characterized etiologic factors of human breast cancer. Epidemiologic studies on Japanese atomic bomb survivors and on clinically irradiated patients established that female breast is among the most susceptible organs to radiation-induced cancers.¹⁴)

Animal models of radiation carcinogenesis have many advantages in providing information about radiation-associated human cancer risk. First, they are the only measure for estimating radiation-associated cancer risk when epidemiologic evidence is lacking. For example, animal experiments are essential to estimate the carcinogenic effect of neutron radiation and nuclear fuel materials.⁵,⁶) Second, since randomized human studies are impossible, animal experiments provide complementary information where epidemiologic studies suffer from biases and confounding factors. For instance, in the studies on Japanese atomic bomb survivors, estimation of the modifying effect of age at exposure on radiation-associated cancer risk is complicated by the chronologic changes in the background cancer incidence in some cancers; thus, cancer incidence data of the childhood exposure population are compared to the most recent incidence data, while data of the adulthood exposure group are compared to old data.⁴) Third, mechanistic understandings are essential for interpretation of epidemiologic observations, and this is provided by animal models, which are usually well defined with respect to genetic and environmental conditions.

Rodent models play an important role in understanding the natural history, mechanism, and modifying factors of breast cancer development. As discussed elsewhere,⁷) since human breast cancer is heterogeneous at the morphological, genetic and molecular levels, any given animal model could not mimic the spectrum of human breast cancers, and animal models could mimic, at best, major subsets and pathways. In

¹Corresponding author: Phone: 043-206-3160, Fax: 043-206-4138, E-mail: timaoka@nirs.go.jp, yshimada@nirs.go.jp
²Experimental Radiobiology for Children’s Health Research Group, Research Center for Radiation Protection, National Institute of Radiological Sciences, Japan; ³Department of Molecular Radiobiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Japan.

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this respect, rodent models provide a variety of breast cancer
types, as reviewed below, and can be used for numerous
types of studies including assays of putative oncogenic and
chemopreventative agents, pathogenesis due to specific
treatment regimens or genetic alterations, etc. Two classes of
rodent models have been used frequently: genetically mod-
ified mouse models and chemically induced carcinogenesis
models. The radiation-induced model, nevertheless, is
potentially very important, since radiation exposure is one of
the very few epidemiologically proven etiologic factors of
human breast cancer. As reviewed here, animal models of
radiation carcinogenesis sometimes give us observations
that do not hold in other models. We focus on the difference
between radiation- and chemically induced mammary car-
cinogenesis, with an emphasis on rat models, and attempt to
clarify the future role of animal models of radiation-induced
mammary cancer.

MODELS OF RADIATION-INDUCED
BREAST CANCER

Reported models of radiation-induced mammary car-
cinogenesis in rodents are summarized in Table 1. The rat is a
widely used model to study the risk and mechanism of breast
carcinogenesis because rat mammary cancers are compara-
table to human breast cancers in many respects, such as high
frequency of hormone dependence and, pathologically, pro-
gression from ductal hyperplasia and ductal carcinoma in
situ. More than fifty years have passed since induction of
mammary cancer by a single X-ray irradiation of the
Sprague-Dawley rat was first reported. Until recently, the
Sprague-Dawley rat, as well as several Wistar-related
strains (Wistar Furth, WAG, WM, and Lewis) have been
used in most studies. Although the use of other strains such as
the F344 strain has been documented, comparative
studies have revealed that the Sprague-Dawley strain is
most susceptible to radiation-induced mammary car-
cinogenesis. The ACI rat is a unique model in which
mammary cancer is induced by estrogen treatment, which
can synergize with cancer induction by ionizing radiation.

The mouse provides an indispensable model system in
which the effects of gene manipulation can be studied in
vivo. Although mouse mammary tumors do have some dis-
similarities from human breast cancers, such as the low
frequency of hormone dependence and the progression of
carcinoma predominantly from alveolar hyperplasia, they
provide a valuable route for genetic experimentation.
BALB/c mice have been used frequently in radiation car-
cinogenesis studies. The BALB/c strain is known to
harbor a unique functional polymorphism of the Prkdc
(DNA-dependent protein kinase catalytic subunit) gene.
Heterozygous mutant mice of the tumor suppressor gene
Tp53, in the genetic background of BALB/c, develop
mammary cancer at a low frequency and the incidence
increases after radiation exposure. The induction is fur-
ther increased by hemizygosity for Atm. Double hetero-
ygous mice for Brca1 and Tp53 also develop mammary
cancer after irradiation. Mutant mouse strains for Apc
(adenomatous polyposis coli) show increased incidence
of mammary tumors after irradiation, though these tumors
show different histopathologic characteristics from human
breast carcinoma.

In addition to sparsely ionizing (low linear energy transfer
[LET]) radiations such as photon (X- and γ-ray) radiation,
induction of mammary cancers is observed after adminis-
tration of densely ionizing (high LET) particle radiations
including neutrons and heavy ions. Rat and mouse models
have provided important information on the risk of
neutrons and heavy ions including neon, iron, and carbon ions. These studies have indicated the high
relative biologic effectiveness of high-LET radiation for
mammary cancer induction. Mammary cancer development
following administration of plutonium has also been docu-
mented.

To briefly summarize chemically induced mammary car-
cinogenesis models, BALB/c, FVB, and other strains of
mouse are frequently used for mammary cancer induction
by two classes of carcinogens: polyaromatic hydrocarbons
(methyleneolanthrene, 1,2,5,6-dibenzanthracene, and 7,12-
dimethylbenz(a)anthracene [DMBA]) and alkylating agents
(1-methyl-1-nitrosourea [MNU], 1-ethyl-1-nitrosourea [ENU], and urethane). Rat mammary cancers are usually induced
by a single, high-dosage treatment with DMBA or MNU.
Also used recently is a protocol in which rats are treated
repeatedly with heterocyclic amines such as 2-amino-1-
methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a represen-
tative food-borne carcinogen. Sprague-Dawley and F344
rats are among the most frequently used rat strains in these
studies.

| Species | Strain/genotype | Type of radiation |
|---------|----------------|------------------|
| Rat     | Sprague-Dawley | Photon, neutron, heavy ions, ²³⁹Pu |
| Wistar (Wistar-Furth, WAG, WM, Lewis) | ACI | Photon, neutron, ²³⁹Pu |
| ACI     | BALB/c         | Photon, neutron |
| Mouse   | BALB/c Tp53⁻/+ | Photon |
| BALB/c Tp53⁻/⁻ Atm⁻/⁻ | Brcal⁻/⁻ Tp53⁻/⁻ (strain unspecified) | Photon |
| C57BL/6 Apo⁻/⁻, Apo⁻/⁻/⁻Prkdc⁻/⁻ | Balb/c | Photon |
PATHOLOGY AND NATURAL HISTORY

Extensive descriptions of the pathology of radiation- and chemically induced rodent mammary tumors have been documented in several excellent reviews.\textsuperscript{45,47,48} Very briefly, development of benign mammary tumors such as fibroadenoma is prominent after irradiation of rats, although significant development of adenocarcinoma (malignant tumor) is observed.\textsuperscript{49} In comparison, chemical carcinogens tend to induce mainly adenocarcinoma.\textsuperscript{45} To date, no pathologic differences between chemically induced and radiation-induced adenocarcinomas have been documented.\textsuperscript{55}

Temporal histopathologic changes leading to the development of carcinoma in the chemical carcinogenesis model have been extensively studied.\textsuperscript{9} The mammary gland consists of epithelial tissue of the mammary ducts embedded in fatty stromal tissue (Fig. 1A). In the course of normal organogenesis, the mammary gland rapidly grows during and after puberty, when mammary ducts elongate and bifurcate to fill the subcutaneous mammary fat pad (Fig. 1B). This is achieved by a controlled balance of cell proliferation and death within the terminal end bud (TEB), the club-shaped structure at the growing ductal terminus, which is present from prepubertal to postpubertal stages (Fig. 1C).\textsuperscript{50} Differentiated structures such as terminal ducts and alveolar buds are formed along the duct (Fig. 1C) and, as the gland attains full development, TEBs also regress and differentiate into one of these structures.\textsuperscript{51} Many lines of evidence suggest that the TEB is the major target structure of chemical carcinogens. After treatment of rats with chemical carcinogens, TEBs show pathological changes such as delayed regression, high proliferation index, and consequent development of hyperplastic and premalignant ductal lesions.\textsuperscript{52–54}

Information regarding radiation-induced carcinogenesis is relatively limited. In mice and rats, pyknotic nuclear aberration and suppressed cell proliferation have been observed in TEBs at 6–24 hours after irradiation, which return to normal levels by 1.5–3 days.\textsuperscript{55,56} Delayed regression of TEBs, with sustained cell proliferation, and development of ductal hyperplasia are seen 4–8 weeks after irradiation of rats (Fig. 2).\textsuperscript{55} These changes are similar to those observed after stimulation with chemical carcinogens, as mentioned above. Hyperplastic alveolar nodules (HANs) are induced by X-irradiation in rats,\textsuperscript{57} though they are not considered to be precursor lesions of rat mammary carcinoma in chemical induction models.\textsuperscript{59} Thus, evidence suggests that the pathogenesis of radiation-induced mammary carcinogenesis may be largely similar to that of chemical carcinogenesis.

GENETIC CHANGES IN CANCER

One characteristic property of ionizing radiation is that it produces DNA double strand breaks, in addition to other oxidative damage; as a consequence, deletions and discontinuous loss of heterozygosity (LOH) are a signature of the mutagenic action of radiation.\textsuperscript{58} On the other hand, carcinogenic chemicals used to induce mammary cancer generally generate adducts to DNA and result in base substitutions and small deletions.\textsuperscript{59}

Base substitution mutations (especially at codons 12 and 13) in the H-ras proto-oncogene are frequently seen in...
MNU-induced rat mammary cancer\(^{60}\) and to a lesser extent in PhIP-induced ones\(^{61-63}\) but not in DMBA-induced cancers.\(^{64}\) Rat mammary cancers induced by \(\gamma\) rays and heavy ions harbor no mutation of \(H\text{-}ras\)\(^{29,65}\) as expected from their preferential induction of deletions and LOH. \(H\text{-}ras\) mutation is thus unlikely to be a causative event in radiation-induced rat mammary carcinogenesis.

LOH is one of the mechanisms through which tumor suppressor genes are inactivated in cancer cells. Searching for LOH regions in cancer, therefore, is generally a promising strategy to discover important tumor suppressor genes. Several LOH regions have been found in PhIP-induced rat mammary cancers\(^{66,67}\) whereas LOH is a rare event in DMBA-, MNU-, and radiation-induced rat mammary cancers.\(^{20,68}\) The low frequency of LOH in radiation-induced mammary cancer is surprising considering the ability of ionizing radiation to induce LOH in other studies.\(^{58,69,70}\)

Comparative genomic hybridization is a powerful tool to detect amplification and deletion of chromosomal regions and has been used to study PhIP- and DMBA-induced rat mammary cancers.\(^{71}\) This analysis has revealed that amplification of several chromosomal regions is characteristic to PhIP-induced cancer, whereas such copy number aberrations are absent in DMBA-induced cancers.\(^{71}\) Studies on radiation-induced cancer are awaited.

Comprehensive gene expression profiling using microarrays is a warranted strategy to dissect important gene expression changes. Rat mammary cancers induced by ionizing radiation, PhIP, DMBA, MNU, and other chemical carcinogens, as well as spontaneously arising mammary cancers, have been analyzed in this manner.\(^{72-77}\) Many of these studies have questioned if cancers of different etiological origins exhibit different gene expression patterns. The results indicate the existence of some differences in gene expression patterns; however, the expression of most genes seems to be similar between cancers of different etiological origins.

Epigenetic events such as alteration of DNA methylation at CpG islands have not been extensively studied in rodent mammary cancer models, and remain an open and promising area of research. Taken together, evidence so far suggests that the genetic alterations may be different between radiation and chemical carcinogenesis models, but the resulting alterations in gene expression are largely similar.

**INDIRECT EFFECTS OF CARCINOGENS**

Although direct genetic alteration is believed to be the principal role of ionizing radiation in carcinogenesis, evidence suggests the existence of some other effects. An in vitro study has suggested the effect of irradiated human mammary fibroblasts on co-cultured non-irradiated mammary epithelial cells to disrupt normal morphogenesis of epithelial ducts.\(^{78}\) An in vivo study has revealed that irradiated mouse mammary stroma has the ability to transform a non-tumorigenic mammary cell line to a tumorigenic state upon transplantation of the cells into stroma.\(^{79}\) Stroma-derived transforming growth factor \(\beta\) (TGF\(\beta\)) may be involved in mediating such indirect effects of ionizing radiation. Evidence indicates that irradiation induces chronic activation of TGF\(\beta\) in the fatty stroma of the mouse mammary gland\(^{80,81}\) and may cause remodeling of the stromal extracellular matrix.\(^{81}\) Activated TGF\(\beta\) may also translocate to the epithelial tissue\(^{80,81}\) and mediate p53-dependent radiation response and epithelial-mesenchymal transition.\(^{82,83}\) In chemical carcinogenesis, a similar indirect (stroma-mediated) effect of MNU has been documented in a rat experiment in which non-treated mammary epithelium gives rise to cancer after being transplanted into the mammary stromal fat of MNU-treated rats.\(^{84}\) In contrast, the stroma is not a major target in DMBA-mediated tumorigenesis of mouse mammary preneoplastic cells.\(^{85}\) These opposing lines of evidence may indicate differences in the mode of action of the carcinogens, differences between species (mouse vs. rat), or differences in experimental design. Because more information is currently available for radiation carcinogenesis models, future comparative studies using chemical induction models could offer important clues to unveiling a more complete picture of such indirect carcinogenic effects.
RISK MODIFICATION BY REPRODUCTIVE FACTORS

Human breast cancer risk is positively associated with obesity (high body mass index) as well as reproduction-related risk factors such as early age at menarche, late age at first full-term pregnancy, and late age at menopause, all of which are related to a prolonged period of endogenous estrogen exposure. A study of atomic bomb survivors indicated that many of these factors, as well as estrogen use, modify the radiation-associated risk of breast cancer in women. Estrogen-related modification of mammary cancer induction is thus an important issue in animal models.

Estrogen receptor (ER) expression of tumors is associated with the estrogen responsiveness of tumors. Whereas non-ovariectomized rats develop ER-positive mammary cancers after irradiation, prepubertally ovariectomized rats, irradiated at adulthood, develop ER-negative mammary cancer, albeit at a low incidence. Similarly, most mammary carcinomas that develop in prepubertal MNU-treated rats are ER-positive, whereas ER-negative carcinoma develops at a low incidence shortly after MNU treatment. Most DMBA-, MNU-, and radiation-induced rat mammary cancers undergo regression after ovariectomy and are thus ovary dependent. These results are consistent in that the estrogen/ER signaling plays pivotal roles in promotion/progression and maintenance of most radiation- and chemically induced rat mammary cancers.

Regarding the risk modification by parity, pregnancy reduces the incidence of chemically induced rat mammary cancer whether carcinogen is administered before, during, or after pregnancy. This protective effect of parity may be due to pregnancy-associated changes in the systemic hormonal environment, the mammary gland content of hormone-responsive cells, and the initial responsiveness of the mammary cells to carcinogens. In contrast, pregnancy after irradiation does not affect the incidence of rat mammary cancer. The incidence of mammary cancer in rats irradiated during pregnancy, lactating, or post-lactating stages is no different from that in age-matched virgins. When virgin, pregnant, and lactating rats are irradiated and then subjected to long-term estrogen treatment (i.e., identical promotional environment), the lactating rat is by far the most susceptible, while the virgin is most resistant. Further studies such as transplantation experiments may be needed to distinguish between these putative initiation- and hormone-related effects of parity on radiation carcinogenesis.

RISK MODIFICATION BY AGE AT EXPOSURE

One important issue in risk assessment of carcinogens is the modifying effect of age at time of exposure. A surprisingly high incidence of early-onset breast cancer is observed in atomic bomb survivors who were exposed in childhood. Several studies have addressed this issue using both rat and Apc(Min/+) mouse models of radiation-induced mammary carcinogenesis. Though the mechanism is not understood, the carcinogenic effect of prepubertal radiation exposure is small, the effects of postpubertal and adulthood exposures are larger, and the effect in old (> 60 weeks) animals is low. This pattern is different from the narrow window of the susceptible period in chemically induced rat mammary carcinogenesis. Since the high susceptibility of postpubertal rats to DMBA does not exactly coincide with the number of TEBs or their proliferation index, this window of susceptibility is understood in association with the transitional differentiation state of TEBs into alveolar buds around this age. In addition, the low level of DMBA-DNA adducts in prepubertal rats may
indicate weak metabolic activation of DMBA into its carcinogenic form in immature mammary gland. Administration of MNU to prepubertal rats is more effective in inducing mammary cancer than that to postpubertal rats, and the susceptibility decreases with age thereafter (Fig. 3B).\(^{109,110}\) This age dependence of MNU is explained by deficiency of a DNA repair enzyme in immature rat mammary epithelial cells.\(^{111}\) A similar age dependence is observed for ENU-induced mammary carcinogenesis of Apc\(^{Min/+}\) mice (Fig. 3B).\(^{112}\) The carcinogenic effect of PhIP is higher in pubertal rats than in mature animals (Fig. 3B).\(^{113}\) This modifying effect of age does not correlate with either PhIP-DNA adduct levels or PhIP-induced mutation frequency, but is only explained by age-dependent gene expression changes induced by PhIP administration.\(^{113}\) These studies suggest that, in mammary cancer induction, the modifying effect of age at exposure and its underlying mechanism are largely dependent on the carcinogen species.

**MAMMARY TARGET CELLS OF CARCINOGENS**

The mammary epithelium contains small fractions of stem cells (which are able to reconstitute the whole gland) and progenitor cells (with partial regenerative potency) plus a large population of differentiated cells.\(^{114,115}\) The existence of mammary stem cells was first suggested by the complete regeneration of mammary gland after inoculation of isolated mammary epithelial cells into subcutaneous fat pads of syngeneic rats.\(^{116}\) The regenerative activity of the rat mammary stem/progenitor cells is measured by inoculating dispersed mammary epithelial cells into the mammary fat pad of other rats and analyzing any resulting formation of colonies.\(^{114}\) The unit of colony formation therein is termed a clonogen and is thought to represent individual stem and/or progenitor cells. In fact, these colonies contain cells that produce morphologically different (i.e., ductal and alveolar) colonies upon subsequent transplantation, indicating the stem cell-like property of clonogenic cells.\(^{117}\) A significant number of clonogens survive after irradiation at a carcinogenic dose, and cancer initiation is calculated to occur frequently in surviving clonogens.\(^{118-120}\) These observations suggest that mammary stem/progenitor cells that survive radiation exposure may initiate cancer development. Indeed, recent studies have identified a population of mouse mammary stem cells (CD49\(^{hi}\)/CD24\(^{med}\) or CD29\(^{hi}\)/CD24\(^{hi}\))\(^{121,122}\) that are sensitive to a high dose (4 Gy) of radiation,\(^{123}\) whereas a putative progenitor cell population (CD29\(^{hi}\)/CD24\(^{-}\)/Sca-1\(^{hi}\)) is radioresistant and enriched 24 hours after irradiation.\(^{123}\)

The generative feature of the TEB, producing all mammary epithelial structures, strongly suggests the existence of stem cells in the TEB; however, the fact that transplantation of any portion of the gland parenchyma generates a complete gland also indicates their existence throughout the gland.\(^{124}\) As mentioned above, mammary carcinogenesis induced by acute postpubertal stimulation is likely to originate mainly from TEBs. Because the TEB consists of proliferating, undifferentiated cells,\(^{50}\) the target cell type of carcinogens may be stem or progenitor cells, which are abundant in this structure. However, since TEBs disappear once the postpubertal mammary gland development is complete, they do not seem to be important in spontaneous mammary cancer development in non-treated animals or in cancer induction by long-term or adulthood carcinogenic treatment. A careful observation of MNU-induced premalignant lesions that are disseminated throughout the gland\(^{125}\) also implies that some cells outside TEBs are involved in cancer initiation.

Cancer development in humans may be a result of accumulation of mutations over a long period of time. This assumption strongly suggests that the tissue stem/progenitor cell, which has a long lifespan, is the cell type from which cancer arises. Detection of long-term label-retaining cells is one criterion to indicate the existence of long-lived cells in vivo. In the mouse mammary gland, radiolabeled thymidine, administered to virgin mice, is retained in a subset of cells for more than 6 weeks (even after pregnancy).\(^{125-127}\) A double-labeling experiment has suggested that the radiolabeled DNA molecules are retained in the cells through preferential retention of the template DNA strands within the long-lived mother cells during chromosomal segregation.\(^{125}\) Long-lived (9 weeks), label-retaining cells of the mouse mammary gland contain enriched Sca-1\(^{hi}\) cells, a putative progenitor cell population.\(^{128}\) In experimental carcinogenesis, fractionation and protraction of irradiation is a unique method to induce mutations selectively in long-lived cells. Protracted irradiation over a period of 16–28 weeks is known to induce rat mammary cancer at a level comparable to that after a single irradiation at the same total dose.\(^{129,130}\) This observation indicates that the long-lived cell population is an important target cell type in radiation carcinogenesis, even if one takes into account the repair of damage after each fractionated irradiation.

In the rat model of mammary cancer induction by a single postpubertal irradiation, a short-term estrogen treatment during a period around irradiation increases the incidence of mammary cancer in rats\(^{24,28,131}\) and treatment with an ER antagonist reduces the incidence.\(^{132}\) Incidence of radiation-induced rat mammary cancer is also decreased by temporary hormonal ablation by ovariectomy prior to irradiation, followed by chronic estrogen supplementation after irradiation.\(^{58,89}\) This reduction is recovered by estrogen treatment immediately after ovariectomy, but not by progesterone or prolactin. These lines of evidence suggest the involvement of estrogen-responsive cells in cancer initiation. Interestingly, in rats treated chronically with estrogen, protraction of irradiation over a period greater than 8 weeks diminishes the radiation-associated mammary cancer risk that is otherwise evident at the same total dose.\(^{130}\) This estrogen-dependent protraction effect is explained by damage repair.
in estrogen-responsive cells or, more attractively, the high susceptibility of estrogen-responsive target cells with a lifespan less than 8 weeks. Indeed, steroid hormone receptor expression is absent in mouse mammary stem cells isolated by surface markers and in long-lived (9 weeks) label-retaining cells, whereas ER-expressing cells are enriched in label-retaining (7 weeks) cells of estrogen-stimulated mice.

Taken together, it is postulated that, upon acute high-dose irradiation, most mammary stem cells may be killed and progenitor cells with induced mutations may proliferate thereafter. These progenitor cells may have a long life (in nature or by taking the evacuated stem cell niche), accumulate further mutations, and finally give rise to cancer. During repeated low-dose exposures, both stem and progenitor cells may survive and accumulate mutations. Estrogen may increase the number of estrogen-responsive progenitor cells that can be targeted by carcinogens. This working hypothesis must be challenged by further investigation. Identification of the target cell type has an important meaning for risk assessment, in that an infinite lifespan of the target cell would permit accumulation of mutations over the lifetime of the individual, and necessitate long-term management of exposure history.

**PERSPECTIVE AND CONCLUSION**

Animal models of radiation carcinogenesis are valuable tools to study underlying mechanisms of relevant human carcinogenesis. Among recent progresses in breast cancer research is the finding that human breast cancers are subdivided into the following five subtypes based on characteristic gene expression: luminal A, luminal B, HER2 (human epidermal growth factor receptor 2)-positive, normal-like, and basal-like. Human breast cancer of the basal-like subtype shows triple negativity for ER, progesterone receptor, and HER2, but is positive for expression of basal markers such as cytokeratin 5/6. It is noteworthy that mammary gland stem cells of mice show basal-like phenotypes. Also interesting is the observation that many of the human breast cancers that develop after radiation therapy are associated with basal-like phenotypes. Recently established mouse models of basal-like breast cancer and yet-to-be-established carcinogenic induction models of these subtypes would provide valuable information on the mechanism underlying development of carcinogen-induced breast cancer and the relevance of the stem cell population as a target of carcinogens.

The evidence reviewed herein indicates that radiation and chemical carcinogenesis models of mammary cancer share certain characteristics, although some differences do exist. The cancers in these models exhibit mostly similar gene expression profiles, albeit with some differences.

| Feature                          | Radiation | Chemical |
|---------------------------------|-----------|----------|
| Epidemiology                    | Abundant  | Scarce   |
| Pathology                       | Adenocarcinoma, fibroadenoma | Mainly adenocarcinoma |
| Genetic changes                 |           |          |
| H-ras mutation                  | No        | Yes (MNU, PhIP); no (DMBA) |
| LOH                             | Rare      | Frequent (PhIP); rare (DMBA, MNU) |
| Transcriptome                    | Changed   | Changed  |
| Indirect effect                 | Possible  | Possible |
| Risk modification               |           |          |
| Estrogen                        | Increases risk | Increases risk |
| Parity                          | Not protective | Protective |
| Susceptible age                 | Postpubertal | Postpubertal (DMBA); prepubertal (MNU); pubertal (PhIP) |
| Target structure                | TEB, non-TEB | TEB (DMBA, MNU), non-TEB (MNU) |

* Abbreviations. DMBA, 7,12-dimethylbenz(a)anthracene; LOH, loss of heterozygosity; MNU, 1-methyl-1-nitrosourea; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; TEB, terminal end bud.
but they may have distinct genetic alterations, as observed in the characteristic occurrence of H-\textit{ras} mutations in MNU-induced cancer and LOH regions in PhIP-induced cancer. The existence of indirect effects of carcinogens is suggested for both ionizing radiation and MNU models, though the identity of these indirect effects is uncertain. Estrogens may play a crucial role in the development of cancer in both radiation and chemical models, but the protective effect of parity seems weaker for radiation. Specifically, ionizing radiation may have a strong impact on cancer initiation in a lactating gland. Though only partial evidence is available, ionizing radiation and some chemical carcinogens such as MNU may target stem/progenitor cells located at the TEB or throughout the gland. We perceive that the animal model of radiation carcinogenesis will continue to play a crucial role in bridging results of in vivo animal experiments and observations from human studies and translate into a better understanding of mammary carcinogenesis.

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**REFERENCES**

1. Thompson, D. E., Mabuchi, K., Ron, E., Soda, M., Tokunaga, M., Ochikubo, S., Sugimoto, S., Ikeda, T., Terasaki, M., Izumi, S., et al. (1994) Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. Radiat. Res. \textbf{137}: S17–S67.

2. Ronckers, C. M., Erdmann, C. A. and Land, C. E. (2004) Radiation and breast cancer: a review of current evidence. Breast Cancer Res. \textbf{7}: 21–32.

3. Preston, D. L., Mattsson, A., Holmberg, E., Shore, R., Hildreth, N. G. and Boice, J. D. J. (2002) Radiation effects on breast cancer risk: a pooled analysis of eight cohorts. Radiat. Res. \textbf{158}: 220–235.

4. Preston, D. L., Ron, E., Tokuoka, S., Funamoto, S., Nishi, N., Soda, M., Mabuchi, K. and Kodama, K. (2007) Solid cancer incidence in atomic bomb survivors: 1958–1998. Radiat. Res. \textbf{168}: 1–64.

5. Broerse, J. J., van Bekkum, D. W., Zoetelief, J. and Zurcher, C. (1991) Relative biological effectiveness for neutron carcinogenesis in monkeys and rats. Radiat. Res. \textbf{128}: S128–S135.

6. Luz, A., Muller, W. A., Linzner, U., Strauss, P. G., Schmidt, J., Muller, K., Atkinson, M. J., Murray, A. B., Gossner, W., Erfve, V., et al. (1991) Bone tumor induction after incorporation of short-lived radionuclides. Radiat. Environ Biophys \textbf{30}: 225–227.

7. Medina, D. and Thompson, H. J. (2000) A comparison of the salient features of mouse, rat, and human mammary tumorogenesis. In Methods in mammary gland biology and breast cancer research. (Eds.) Ip, M. M. and Asch, B. B., pp. 31–36. Kluwer Academic/Plenum Publishers, New York.

8. Medina, D. (2000) Mouse models for mammary cancer. In Methods in mammary gland biology and breast cancer research, (Eds.) Ip, M. M. and Asch, B. B., pp. 3–17. Kluwer Academic/Plenum Publishers, New York.

9. Thompson, H. J. and Singh, M. (2000) Rat models of premalignant breast disease. J. Mammary Gland Biol. Neoplasia \textbf{5}: 409–420.

10. Nandi, S., Guzman, R. C. and Yang, J. (1995) Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. Proc. Natl. Acad. Sci. U. S. A. \textbf{92}: 3650–3657.

11. Gould, M. N. (1995) Rodent models for the study of etiology, prevention and treatment of breast cancer. Semin Cancer Biol. \textbf{6}: 147–152.

12. Finerty, J. C., Binhammer, R. T., Schneider, M. and Cunningham, A. W. (1953) Neoplasms in rats exposed to single-dose total-body X radiation. J. Natl. Cancer Inst. \textbf{14}: 149–157.

13. Shellabarger, C. J., Cronkite, E. P., Bond, V. P. and Lippincott, S. W. (1957) The occurrence of mammary tumors in the rat after sublethal whole-body irradiation. Radiat. Res. \textbf{6}: 501–512.

14. Jacrot, M., Mouri quand, J., Mouri quand, C. and Saez, S. (1979) Mammary carcinogenesis in Sprague-Dawley rats following 3 repeated exposures to 14. 8 MeV neutrons and steroid receptor content of these tumor types. Cancer Lett. \textbf{47}: 143–153.

15. Welsch, C. W., Goodrich-Smith, M., Brown, C. K., Migl orie, N. and Clif forton, K. H. (1981) Effect of an estrogen antagonist (tamoxifen) on the initiation and progression of gamma-irradiation-induced mammary tumors in female Sprague-Dawley rats. Eur. J. Cancer Clin. Oncol. \textbf{17}: 1255–1258.

16. Gragtmans, N. J., Myers, D. K., Johnson, J. R., Jones, A. R. and Johnson, L. D. (1984) Occurrence of mammary tumors in rats after exposure to tritium beta rays and 200-kVp X rays. Radiat. Res. \textbf{99}: 636–650.

17. Mandybur, T. I., Ormsby, I., Samuels, S. and Mancardi, G. L. (1985) Neural, pituitary, and mammary tumors in Sprague-Dawley rats treated with X irradiation to the head and N-ethyl-N-nitrosourea (ENU) during the early postnatal period: a statistical study of tumor incidence and survival. Radiat. Res. \textbf{101}: 460–472.

18. Lemon, H. M., Kumar, P. F., Peterson, C., Rodriguez-Sierra, J. F. and Abbo, K. M. (1989) Inhibition of radiogenic mammary carcinoma in rats by estriol or tamoxifen. Cancer \textbf{63}: 1685–1692.

19. Kantorowitz, D. A., Thompson, H. J. and Furmanski, P. (1995) Effect of high-dose, fractionated local irradiation on MNU-induced carcinogenesis in the rat mammary gland.

J. Radiat. Res., Vol. 50, No. 4 (2009); http://jrr.jstage.jst.go.jp
Carcinogenesis 16: 649–653.

20. Haag, J. D., Hsu, L. C., Newton, M. A. and Gould, M. N. (1996) Allelic imbalance in mammary carcinomas induced by either 7,12-dimethylbenz(a)anthracene or ionizing radiation in rats carrying genes conferring differential susceptibilities to mammary carcinogenesis. Mol. Carcinog 17: 134–143.

21. Bartstra, R. W., Bentvelzen, P. A., Zoetelief, J., Mulder, A. H., Broerse, J. J. and van Bekkum, D. W. (1998) Induction of mammary tumors in rats by single-dose gamma irradiation at different ages. Radiat. Res. 150: 442–450.

22. Inano, H., Suzuki, K., Ishii-Ohba, H., Ikeda, K. and Wakabayashi, K. (1991) Pregnancy-dependent initiation in tumorigenesis of Wistar rat mammary glands by 60Co-irradiation. Carcinogenesis 12: 1085–1090.

23. Shellabarger, C. J. (1972) Mammary neoplastic response of Lewis and Sprague-Dawley female rats to 7,12-dimethylbenz(a)anthracene or x-ray. Cancer Res. 32: 883–885.

24. Holtzman, S., Stone, J. P. and Shellabarger, C. J. (1979) Synergism of diethylstilbestrol and radiation in mammary carcinogenesis in female F344 rats. J. Natl. Cancer Inst. 63: 1071–1074.

25. Clifton, K. H., Yasukawa-Barnes, J., Tanner, M. A. and Haning, R. V. Jr. (1985) Irradiation and prolactin effects on rat mammary carcinogenesis: intrasplenic pituitary and estrone capsule implants. J. Natl. Cancer Inst. 75: 167–175.

26. Vogel, H. H. Jr. and Turner, J. E. (1982) Genetic component in rat mammary carcinogenesis. Radiat. Res. 89: 264–273.

27. Shellabarger, C. J. (1976) Modifying factors in rat mammary gland carcinogenesis. In Biology of Radiation Carcinogenesis. (Eds.) Yuhas, J. M., Tennant, R. W. and Regan, J. D., pp. 31–43. Raven Press, New York.

28. Shellabarger, C. J., Stone, J. P. and Holtzman, S. (1978) Rat differences in mammary tumor induction with estrogen and neutron radiation. J. Natl. Cancer Inst. 61: 1505–1508.

29. Imaoka, T., Nishimura, M., Kakinuma, S., Hatanou, Y., Ohmachi, Y., Kawano, A., Maekawa, A. and Shimada, Y. (2007) High relative biologic effectiveness of carbon ion radiation on induction of rat mammary carcinoma and its lack of H-ras and Tp53 mutations. Int. J. Radiat. Oncol. Biol. Phys. 69: 194–203.

30. Holtzman, S., Stone, J. P. and Shellabarger, C. J. (1981) Synergism of estrogens and X-rays in mammary carcinogenesis in female ACI rats. J. Natl. Cancer Inst. 67: 455–459.

31. Ullrich, R. L. and Preston, R. J. (1991) Radiation induced mammary cancer. J. Radiat. Res. (Tokyo) 32 Suppl 2: 104–109.

32. Yu, Y., Okayasu, R., Weil, M. M., Silver, A., McCarthy, M., Zabriskie, R., Long, S., Cox, R. and Ullrich, R. L. (2001) Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the Prkdc (DNA-dependent protein kinase catalytic subunit) gene. Cancer Res. 61: 1820–1824.

33. Mori, N., Yamate, J., Umesako, S., Hong, D. P., Okumoto, M. and Nakao, R. (2003) Preferential induction of mammary tumors in p53 hemizygous BALB/c mice by fractionated irradiation of a sub-lethal dose of X-rays. J. Radiat. Res. (Tokyo) 44: 249–254.

34. Backlund, M. G., Trasti, S. L., Backlund, D. C., Cressman, V. L., Godfrey, V. and Koller, B. H. (2001) Impact of ionizing radiation and genetic background on mammary tumorigenesis in p53-deficient mice. Cancer Res. 61: 6577–6582.

35. Umesako, S., Fujisawa, K., Iiga, S., Mori, N., Takahashi, M., Hong, D. P., Song, C. W., Haga, S., Imai, S., Niwa, O. and Okimoto, M. (2005) Atm heterozygous deficiency enhances development of mammary carcinomas in p53 heterozygous knockout mouse. Breast Cancer Res. 7: R164–R170.

36. Cressman, V. L., Backlund, D. C., Hicks, E. M., Gowen, L. C., Godfrey, V. and Koller, B. H. (1999) Mammary tumor formation in p53- and BRCA1-deficient mice. Cell Growth Differ 10: 1–10.

37. van der Houwen van Oordt, C. W., Smits, R., Schouten, T. G., Houwing-Duistermaat, J. J., Williamson, S. L., Luz, A., Meera Khan, P., van der Eb, A. J., Breuer, M. L. and Fodde, R. (1999) The genetic background modifies the spontaneous and X-ray-induced tumor spectrum in the Apc1638N mouse model. Genes Chromosomes Cancer 24: 191–198.

38. Imaoka, T., Okamoto, M., Nishimura, M., Nishimura, Y., Ootawara, M., Kakinuma, S., Tokairin, Y. and Shimada, Y. (2006) Mammary tumorigenesis in ApC Mmut mice is enhanced by X-irradiation with a characteristic age dependence. Radiat. Res. 165: 165–173.

39. Ullrich, R. L., Jernigan, M. C. and Storer, J. B. (1977) Neuron carcinogenesis. Dose and dose-rate effects in BALB/c mice. Radiat. Res. 72: 487–498.

40. Ullrich, R. L. (1983) Tumor induction in BALB/c female mice after fission neutron or gamma irradiation. Radiat. Res. 93: 506–515.

41. Ullrich, R. L. (1984) Tumor induction in BALB/c mice after fractionated or protracted exposures to fission-spectrum neutrons. Radiat. Res. 97: 587–597.

42. Pacello, J. F., Christian, A., Cucinotta, F. A., Gridley, D. S., Kathirithamiy, R., Mann, J., Markham, A. R., Moyers, M. F., Novak, G. R., Piantadosi, S., Ricart-Arbona, R., Simonson, D. M., Strandberg, J. D., Vazquez, M., Williams, J. R., Zhang, Y., Zhou, H. and Huso, D. (2004) In vivo mammary tumourigenesis in the Sprague-Dawley rat and microdosimetric correlates. Phys. Med. Biol. 49: 3817–3830.

43. Shellabarger, C. J., Baum, J. W., Holtzman, S. and Stone, J. P. (1985) Neon-20 ion- and X-ray-induced mammary carcinogenesis in female rats. Ann. N. Y. Acad. Sci. 459: 239–244.

44. Sanders, C. L. Jr. (1974) Rat mammary neoplasia following deposition of plutonium. Health Phys. 27: 592–593.

45. Russo, J., Russo, I. H., Rogers, A. E., van Zwieten, M. J. and Gusterson, B. (1990) Tumor of the mammary gland. In Pathology of Tumours in Laboratory Animals Volume 1 - Tumours of the Rat, 2nd Ed. edition, (Eds.) Turusov, V. and Simonson, M., Strandberg, J. D., Vazquez, M., Williams, J. R., Zhang, Y., Zhou, H. and Huso, D. (2004) In vivo mammary tumourigenesis in the Sprague-Dawley rat and microdosimetric correlates. Phys. Med. Biol. 49: 3817–3830.

46. Shellabarger, C. J., Baum, J. W., Holtzman, S. and Stone, J. P. (1985) Neon-20 ion- and X-ray-induced mammary carcinogenesis in female rats. Ann. N. Y. Acad. Sci. 459: 239–244.

47. Sanders, C. L. Jr. (1974) Rat mammary neoplasia following deposition of plutonium. Health Phys. 27: 592–593.

48. Russo, J., Russo, I. H., Rogers, A. E., van Zwieten, M. J. and Gusterson, B. (1990) Tumor of the mammary gland. In Pathology of Tumours in Laboratory Animals Volume 1 - Tumours of the Rat, 2nd Ed. edition, (Eds.) Turusov, V. and Simonson, M., Strandberg, J. D., Vazquez, M., Williams, J. R., Zhang, Y., Zhou, H. and Huso, D. (2004) In vivo mammary tumourigenesis in the Sprague-Dawley rat and microdosimetric correlates. Phys. Med. Biol. 49: 3817–3830.

49. Shellabarger, C. J., Baum, J. W., Holtzman, S. and Stone, J. P. (1985) Neon-20 ion- and X-ray-induced mammary carcinogenesis in female rats. Ann. N. Y. Acad. Sci. 459: 239–244.

50. Sanders, C. L. Jr. (1974) Rat mammary neoplasia following deposition of plutonium. Health Phys. 27: 592–593.
Gland Biol. Neoplasia 5: 187–200.
48. Tsubura, A., Yoshizawa, K., Uehara, N., Yuri, T. and Matsuoka, Y. (2007) Multistep mouse mammary tumorigenesis through pre-neoplasia to neoplasia and acquisition of metastatic potential. Med. Mol. Morphol. 40: 9–17.
49. Shellabarger, C. J., Bond, V. P. and Cronkite, E. P. (1960) Studies on radiation-induced mammary gland neoplasia in the rat. 4. The response of females to a single dose of sublethal total-body gamma radiation as studied until the first appearance of breast neoplasia or death of the animals. Radiat. Res. 13: 242–249.
50. Humphreys, R. C., Krajewska, M., Krasic, S., Jaeger, R., Weiher, H., Krajewski, S., Reed, J. C. and Rosen, J. M. (1996) Apoptosis in the terminal end bud of the murine mammary gland: a mechanism of ductal morphogenesis. Development 122: 4013–4022.
51. Russo, J., Tay, L. K. and Russo, I. H. (1982) Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res. Treat. 2: 5–73.
52. Korkola, J. E. and Archer, M. C. (1999) Resistance to mammary tumorigenesis in Copenhagen rats is associated with the loss of preneoplastic lesions. Carcinogenesis 20: 221–227.
53. Osborne, M. P., Ruperto, J. F., Crowe, J. P., Rosen, P. P. and Teleng, N. T. (1992) Effect of tamoxifen on preneoplastic cell proliferation in N-nitroso-N-methylurea-induced mammary carcinogenesis. Cancer Res. 52: 1477–1480.
54. Shilkaitis, A., Green, A., Steele, V., Lubet, R., Kelloff, G. and Christov, K. (2000) Neoplastic transformation of mammary epithelial cells in rats is associated with decreased apoptotic cell death. Carcinogenesis 21: 227–233.
55. Imaoka, T., Nishimura, M., Nishimura, Y., Kakinuma, S. and Shimada, Y. (2006) Persistent cell proliferation of terminal end buds precedes radiation-induced rat mammary carcinogenesis. In Vivo 20: 353–358.
56. Sharkey, S. M. and Bruce, W. R. (1986) Quantitation of nuclear aberrations as a screen for agents damaging to mammary epithelium. Carcinogenesis 7: 1991–1995.
57. Faulklin, L. J. Jr., Shellabarger, C. J. and DeOme, K. B. (1967) Hyperplastic lesions of Sprague-Dawley rat mammary glands after X irradiation. J. Natl. Cancer Inst. 39: 449–458.
58. Ponomareova, O. N., Rose, J. A., Lasarev, M., Rasey, J. and Turker, M. S. (2002) Tissue-specific deletion and discontinuous loss of heterozygosity are signatures for the mutagenic effects of ionizing radiation in solid tissues. Cancer Res. 62: 1518–1523.
59. Nohmi, T. and Masumura, K. (2005) Molecular nature of intrachromosomal deletions and base substitutions induced by environmental mutagens. Environ Mol. Mutagen 45: 150–161.
60. Sukumar, S., Notario, V., Martin-Zanca, D. and Barbacid, M. (1983) Induction of mammary carcinomas in rats by nitrosomethylurea involves malignant activation of H-ras-1 locus by single point mutations. Nature 306: 658–661.
61. Hokaiwado, N., Asamoto, M., Cho, Y. M., Imaida, K. and Shirai, T. (2001) Frequent c-Ha-ras gene mutations in rat mammary carcinomas induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Cancer Lett. 163: 187–190.
62. Ushijima, T., Kakiuchi, H., Makino, H., Hasegawa, R., Ishizaka, Y., Hirai, H., Yazaki, Y., Ito, N., Sugimura, T. and Nagao, M. (1994) Infrequent mutation of Ha-ras and p53 in rat mammary carcinomas induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Mol. Carcinog 10: 38–44.
63. Yu, M. and Snyderwine, E. G. (2002) H-ras oncogene mutations during development of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced rat mammary gland cancer. Carcinogenesis 23: 2123–2128.
64. Waldmann, V., Suchy, B. and Rabes, H. M. (1993) Cell proliferation and prevalence of ras gene mutations in 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumors. Res. Exp. Med. (Berl) 193: 143–151.
65. Imaoka, T., Nishimura, M., Teramoto, A., Nishimura, Y., Ootawara, M., Osada, H., Kakinuma, S., Maekawa, A. and Shimada, Y. (2005) Cooperative induction of rat mammary cancer by radiation and 1-methyl-1-nitrosourea via the oncogenic pathways involving c-Myc activation and H-ras mutation. Int. J. Cancer 115: 187–193.
66. Toyota, M., Ushijima, T., Weisburger, J. H., Hosoya, Y., Canfian, F., Rivenson, A., Imai, K., Sugimura, T. and Nagao, M. (1996) Microsatellite instability and loss of heterozygosity on chromosome 10 in rat mammary tumors induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Mol. Carcinog 15: 176–182.
67. Yu, M., Ryu, D. Y. and Snyderwine, E. G. (2000) Genomic imbalance in rat mammary gland carcinomas induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Mol. Carcinog 27: 76–83.
68. Haag, J. D., Brasic, G. M., Shepel, L. A., Newton, M. A., Grubbs, C. J., Lubet, R. A., Kelloff, G. J. and Gould, M. N. (1999) A comparative analysis of allelic imbalance events in chemically induced rat mammary, colon, and bladder tumors. Mol. Carcinog 24: 47–56.
69. Shimada, Y., Nishimura, M., Kakinuma, S., Okumoto, M., Shiroishi, T., Clifton, K. H. and Wakana, S. (2000) Radiation-associated loss of heterozygosity at the Zfnl1al (IKaras) locus on chromosome 11 in murine thymic lymphomas. Radiat. Res. 154: 293–300.
70. Yoshida, M. A., Nakata, A., Akiyama, M., Kakinuma, S., Sado, T., Nishimura, M. and Shimada, Y. (2007) Distinct structural abnormalities of chromosomes 11 and 12 associated with loss of heterozygosity in X-ray-induced mouse thymic lymphomas. Cancer Genet. Cytoogenet 179: 1–10.
71. Christian, A. T., Snyderwine, E. G. and Tucker, J. D. (2002) Comparative genominc hybridization analysis of PhIP-induced mammary carcinomas in rats reveals a cytogenic signature. Mutat. Res. 506–507: 113–119.
72. Imaoka, T., Yamashita, S., Nishimura, M., Kakinuma, S., Ushijima, T. and Shimada, Y. (2008) Gene expression profiling distinguishes between spontaneous and radiation-induced rat mammary carcinomas. J. Radiat. Res. (Tokyo) 49: 349–360.
73. Chan, M. M., Lu, X., Merchant, F. M., Iglehart, J. D. and Miron, P. L. (2005) Gene expression profiling of NMU-induced rat mammary tumors: cross species comparison with human breast cancer. Carcinogenesis 26: 1343–1353.
Radiation-Induced Mammary Carcinogenesis 291

74. Shan, L., Yu, M. and Snyderwine, E. G. (2005) Gene expression profiling of chemically induced rat mammary gland cancer. Carcinogenesis 26: 503–509.

75. Shan, L., He, M., Yu, M., Qiu, C., Lee, N. H., Liu, E. T. and Snyderwine, E. G. (2002) cDNA microarray profiling of rat mammary gland carcinomas induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and 7,12-dimethylbenz[a]anthracene. Carcinogenesis 23: 1561–1568.

76. Kuramoto, T., Morimura, K., Yamashita, S., Okochi, E., Watanabe, N., Ohta, T., Ohki, M., Fukushima, S., Sugimura, T. and Ushijima, T. (2002) Etiology-specific gene expression profiles in rat mammary carcinomas. Cancer Res. 62: 3592–3597.

77. Lee, H. J., Lee, Y. J., Kang, C. M., Bae, S., Jeoung, D., Jang, J. J., Lee, S. S., Cho, C. K. and Lee, Y. S. (2008) Differential gene signatures in rat mammary tumors induced by DMBA and those induced by fractionated gamma radiation. Radiat. Res. 170: 579–590.

78. Tsai, K. K., Chuang, E. Y., Little, J. B. and Yuan, Z. M. (2005) Cellular mechanisms for low-dose ionizing radiation-induced perturbation of the breast tissue microenvironment. Cancer Res. 65: 6734–6744.

79. Barcellos-Hoff, M. H. and Ravani, S. A. (2000) Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Res. 60: 1254–1260.

80. Barcellos-Hoff, M. H., Derynck, R., Tsang, M. L. and Weatherbee, J. A. (1994) Transforming growth factor-beta activation in irradiated murine mammary gland. J. Clin. Invest 93: 892–899.

81. Ehrhart, E. J., Segarini, P., Tsang, M. L., Carroll, A. G. and Barcellos-Hoff, M. H. (1997) Latent transforming growth factor beta1 activation in situ: quantitutive and functional evidence after low-dose gamma-irradiation. FASEB J. 11: 991–1002.

82. Ewan, K. B., Henshall-Powell, R. L., Ravani, S. A., Pajares, M. J., Arteaga, C., Warters, R., Akhurst, R. J. and Barcellos-Hoff, M. H. (2002) Transforming growth factor-beta1 mediates cellular response to DNA damage in situ. Cancer Res. 62: 5627–5631.

83. Andarawewa, K. L., Erickson, A. C., Chou, W. S., Costes, S. V., Gascard, P., Mott, J. D., Bissell, M. J. and Barcellos-Hoff, M. H. (2007) Ionizing radiation predisposes nonmalignant human mammary epithelial cells to undergo transforming growth factor beta induced epithelial to mesenchymal transition. Cancer Res. 67: 8662–8670.

84. Maffini, M. V., Soto, A. M., Calabro, J. M., Ucci, A. A. and Sonnenschein, C. (2004) The stroma as a crucial target in rat mammary gland carcinogenesis. J. Cell Sci. 117: 1495–1502.

85. Medina, D. and Kittrell, F. (2005) Stroma is not a major target in DMBA-mediated tumorigenesis of mouse mammary preneoplasia. J. Cell Sci. 118: 123–127.

86. Kelsey, J. L. and Berkowitz, G. S. (1988) Breast cancer epidemiology. Cancer Res. 48: 5615–5623.

87. Goodman, M. T., Cologne, J. B., Moriwaki, H., Vaeth, M. and Mabuchi, K. (1997) Risk factors for primary breast cancer in Japan: 8-year follow-up of atomic bomb survivors. Prev. Med. 26: 144–153.

88. Yamanouchi, H., Ishii-Ohba, H., Suzuki, K., Onoda, M., Wakabayashi, K. and Inano, H. (1995) Relationship between stages of mammary development and sensitivity to gamma-ray irradiation in mammary tumorigenesis in rats. Int. J. Cancer 60: 230–234.

89. Inano, H., Yamanouchi, H., Suzuki, K., Onoda, M. and Wakabayashi, K. (1995) Estradiol-17 beta as an initiation modifier for radiation-induced mammary tumorigenesis of rats ovariectomized before puberty. Carcinogenesis 16: 1871–1877.

90. Knott, K. K., McGinley, J. N., Lubet, R. A., Steele, V. E. and Thompson, H. J. (2001) Effect of the aromatase inhibitor vorozole on estrogen and progesterone receptor content of rat mammary carcinomas induced by 1-methyl-1-nitrosourea. Breast Cancer Res. Treat. 70: 171–183.

91. Thompson, H. J., McGinley, J., Rothhammer, K. and Singh, M. (1998) Ovarian hormone dependence of pre-malignant and malignant mammary gland lesions induced in pre-pubertal rats by 1-methyl-1-nitrosourea. Carcinogenesis 19: 383–386.

92. Ueda, M., Imai, T., Takizawa, T., Onodera, H., Mitsumori, K., Matsu, T. and Hirose, M. (2005) Possible enhancing effects of atrazine on growth of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in ovariectomized Sprague-Dawley rats. Cancer Sci. 96: 19–25.

93. Thompson, H. J., Meeker, L. D., Tagliaferro, A. R. and Becchi, P. J. (1982) Effect of retinyl acetate on the occurrence of ovariain hormone-responsive and -nonresponsive mammary cancers in the rat. Cancer Res. 42: 903–905.

94. Huggins, C. and Fukushima, R. (1963) Cancer in the rat after single exposures to irradiation or hydrocarbons. Age and strain factors. Hormone dependence of the mammary cancers. Radiat. Res. 20: 493–503.

95. Yang, J., Yoshizawa, K., Nandi, S. and Tsbura, A. (1999) Protective effects of pregnancy and lactation against N-methyl-N-nitrosourea-induced mammary carcinomas in female Lewis rats. Carcinogenesis 20: 623–628.

96. Sinha, D. K., Pazik, J. E. and Dao, T. L. (1983) Progression of rat mammary development with age and its relationship to carcinogenesis by a chemical carcinogen. Int. J. Cancer 31: 321–327.

97. Russo, I. H., Koszalka, M. and Russo, J. (1991) Comparative study of the influence of pregnancy and hormonal treatment on mammary carcinogenesis. Br. J. Cancer 64: 481–484.

98. Abrams, T. J., Guzman, R. C., Swanson, S. M., Thordarson, G., Talamantes, F. and Nandi, S. (1993) Changes in the parous rat mammary gland environment are involved in parity-associated protection against mammary carcinogenesis. Anticancer Res. 13: 4115–4121.

99. Thordarson, G., Jin, E., Guzman, R. C., Swanson, S. M., Nandi, S. and Talamantes, F. (1995) Refractoriness to mammary tumorigenesis in parous rats: is it caused by persistent changes in the hormonal environment or permanent biochemical alterations in the mammary epithelia? Carcinogenesis 16: 2847–2853.

100. Sivaraman, L., Stephens, L. C., Markaverich, B. M., Clark, J. A., Knrack, S., Conneely, O. M., O’Malley, B. W. and Medina, D. (1998) Hormone-induced refractoriness to mam-
109. Ariazi, J. L., Haag, J. D., Lindstrom, M. J. and Gould, M. N. 
110. Moser, A. R., Mattes, E. M., Dove, W. F., Lindstrom, M. J., 
111. Russo, J., Wilgus, G. and Russo, I. H. (1979) Susceptibility 
112. Grubbs, C. J., Peckham, J. C. and Cato, K. D. (1983) Mam - 
113. Holtzman, S., Stone, J. P. and Shellabarger, C. J. (1982) 
114. Kamiya, K., Gould, M. N. and Clifton, K. H. (1991) Differ- 
115. Smith, G. H. (1996) Experimental mammary epithelial mor-
116. Kordon, E. C. and Smith, G. H. (1998) An entire functional 
mammary gland may comprise the progeny from a single cell. 
117. Kim, N. D., Oberley, T. D., Yasukawa-Barnes, J. and Clifton, 
118. Gould, M. N. and Clifton, K. H. (2004) Susceptibility of rats to mammary gland carcinogenesis in Wistar-Furth rats. Carcinogenesis 19: 1573–1581. 
119. Clifton, K. H., Tanner, M. A. and Gould, M. N. (1986) Assessment of radiogenic cancer initiation frequency per clongenic rat mammary cell in vivo. Cancer Res. 46: 2390–2395. 
120. Kamiya, K., Yasukawa-Barnes, J., Mitchell, J. M., Gould, M. N. and Clifton, K. H. (1995) Evidence that carcinogenesis involves an imbalance between epigenetic high-frequency initiation and suppression of promotion. Proc. Natl. Acad. Sci. U. S. A. 92: 1332–1336. 
121. Stingl, J., Eirew, P., Ricketson, I., Shackleton, M., Vaillant, F., Choi, D., Li, H. I. and Eaves, C. J. (2006) Purification and unique properties of mammary epithelial stem cells. Nature 439: 993–997. 
122. Shackleton, M., Vaillant, F., Simpson, K. J., Stingl, J., Smyth, G. K., Asselin-Labat, M. L., Wu, L., Lindeman, G. J. and Visvader, J. E. (2006) Generation of a functional mammary gland from a single stem cell. Nature 439: 84–88. 
123. Woodward, W. A., Chen, M. S., Behbod, F., Alfaro, M. P., Buchholz, T. A. and Rosen, J. M. (2007) WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. Proc. Natl. Acad. Sci. U. S. A. 104: 618–623. 
124. Smith, G. H. and Boulanger, C. A. (2003) Mammary epithelial stem cells: transplantation and self-renewal analysis. Cell Prolif 36 Suppl 1: 3–15. 
125. Smith, G. H. (2005) Label-retaining epithelial cells in mouse mammary gland divide asymmetrically and retain their template DNA strands. Development 132: 681–687. 
126. Booth, B. W. and Smith, G. H. (2006) Estrogen receptor-alpha and progesterone receptor are expressed in label-retaining mammary epithelial cells that divide asymmetrically and retain their template DNA strands. Breast Cancer Res. 8: R49. 
127. Booth, B. W., Boulanger, C. A. and Smith, G. H. (2008) Selective segregation of DNA strands persists in long label retaining mammary cells during pregnancy. Breast Cancer Res. 10: R90. 
128. Welm, B. E., Tepera, S. B., Venezia, T., Graubert, T. A., Rosen, J. M. and Goodell, M. A. (2002) Sca-1<sup>low</sup> cells in the mouse mammary gland represent an enriched progenitor cell population. Dev. Biol. 245: 42–56. 
129. Shellabarger, C. J., Bond, V. P., Aponte, G. E. and Cronkite,
E. P. (1966) Results of fractionation and protraction of total-body radiation on rat mammary neoplasia. Cancer Res. 26: 509–513.

130. Bartstra, R. W., Bentvelzen, P. A., Zoetelief, J., Mulder, A. H., Broerse, J. J. and van Bekkum, D. W. (2000) The effects of fractionated gamma irradiation on induction of mammary carcinoma in normal and estrogen-treated rats. Radiat. Res. 153: 557–569.

131. Broerse, J. J., Hennen, L. A., Klapwijk, W. M. and Solleveld, H. A. (1987) Mammary carcinogenesis in different rat strains after irradiation and hormone administration. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 51: 1091–1100.

132. Asselin-Labat, M. L., Shackleton, M., Stingl, J., Vaillant, F., Forrest, N. C., Eaves, C. J., Visvader, J. E. and Lindeman, G. J. (2006) Steroid hormone receptor status of mouse mammary stem cells. J. Natl. Cancer Inst. 98: 1011–1014.

133. Sleeman, K. E., Kendrick, H., Robertson, D., Isacke, C. M., Ashworth, A. and Smalley, M. J. (2007) Dissociation of estrogen receptor expression and in vivo stem cell activity in the mammary gland. J. Cell Biol. 176: 19–26.

134. Sorlie, T., Perou, C. M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Thorsen, T., Quist, H., Mates, J. C., Brown, P. O., Botstein, D., Eystein Lonning, P. and Borresen-Dale, A. L. (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. U. S. A. 98: 10869–10874.

135. Castiglioni, F., Terenziani, M., Carcangi, M. L., Miliano, R., Aiello, P., Bertola, L., Triulzi, T., Gasparini, P., Camerini, T., Sozzi, G., Fossati-Bellani, F., Menard, S. and Tagliaabue, E. (2007) Radiation effects on development of HER2-positive breast carcinomas. Clin. Cancer Res. 13: 46–51.

136. Saal, L. H., Gruvberger-Saal, S. K., Persson, C., Lovgren, K., Jumppanen, M., Staaf, J., Jonsson, G., Pires, M. M., Maurer, M., Holm, K., Koujak, S., Subramaniyam, S., Vallon-Christersson, J., Olsson, H., Su, T., Memeo, L., Ludwig, T., Ethier, S. P., Krogh, M., Szabolcs, M., Murty, V. V., Isola, J., Hibshoosh, H., Parsons, R. and Borg, A. (2008) Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. Nat. Genet. 40: 102–107.

137. Liu, X., Holstege, H., van der Gulden, H., Treur-Mulder, M., Zevenhoven, J., Velds, A., Kerkhoven, R. M., van Vliet, M. H., Wessels, L. F., Peterse, J. L., Berns, A. and Jonkers, J. (2007) Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. Proc. Natl. Acad. Sci. U. S. A. 104: 12111–12116.

138. McCarthy, A., Savage, K., Gabriel, A., Naceur, C., Reis-Filho, J. S. and Ashworth, A. (2007) A mouse model of basal-like breast carcinoma with metaplastic elements. J. Pathol. 211: 389–398.