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Stromal Mediation of Radiation Carcinogenesis

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Abstract

Ionizing radiation is a well-established carcinogen in human breast and rodent mammary gland. This review addresses evidence that radiation elicits the critical stromal context for cancer, affecting not only frequency but the type of cancer. Recent data from the breast tumors of women treated with radiation therapy and the cellular mechanisms evident in experimental models suggest that radiation effects on stromal-epithelial interactions and tissue composition are a major determinant of cancer development.

Keywords

Ionizing radiation; Carcinogenesis; Stromal-epithelial interactions; Mammary gland

Introduction

Many investigators have argued that disruption of the cell interactions and tissue architecture can be a primary driver of carcinogenesis [1–5]. Recent experiments published from Weinberg [6], Moses [7], Sonnenchein [8] and Coussens [9] offer provocative evidence that composition of the microenvironment is a critical determinant of cancer suppression or promotion and highlight multicellular contributions to the carcinogenic response and cancer progression. Over a decade ago, we hypothesized that modulation of the tissue microenvironment is an additional action of radiation specifically, and of carcinogens in general [10]. The prevailing paradigm of cancer risk following radiation focuses on the probability of DNA damage that can lead to mutations in susceptible cells [11]. An alternative hypothesis is that cancer emerges from irradiated tissues as a result of complex, but ultimately predictable, interactions between mutagenesis from DNA damage and radiation effects on the microenvironment and cell interactions is [12]. Just as DNA damage elicits a dramatic transition in signaling within a cell, each irradiated tissue has its own set of signals and composition, distinct from those of unirradiated tissue and different from other irradiated tissues. Biological responses to radiation damage quickly evolve and amplify, mostly in a non-linear manner, and can alter daughter cell fates such as differentiation and senescence [13–16], induce long-range signals that affect non-irradiated cells [17–20], or generate a state of chronic genomic instability [17–21]. The sum of these events, occurring in different organs and highly modulated by genotype, predicates the health risks. To test this hypothesis we created radiation chimeric tissue by transplanting unirradiated, preneoplastic mammary cells to an irradiated mammary gland [10]. The data from this

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model and others discussed below supports the hypothesis that non-mutagenic effects of radiation on the stroma can contribute significantly to radiation carcinogenesis in vivo.

Radiation Exposure and Cancer in Humans

Ionizing radiation is a known carcinogen of humans and experimental models (reviewed in [22]). Excess cancers were observed in the Japanese atomic-bomb survivors at acute radiation doses of 10 to 400 cGy, which are 40 to 1600 times the average yearly background levels in the United States. The excess risks vary significantly with gender, attained age, and age at exposure for all solid cancers as a group and many individual sites as a consequence of radiation from the atomic bomb [23]. It has been estimated that if radiation exposure occurs at age 30, the solid cancer rates at age 70 is increased by about 35% per Gy for men and 58% per Gy for women [23].

A pooled analysis by Preston and colleagues of eight populations exposed to ionizing radiation showed that increased risk of breast cancer is inversely correlated with age at irradiation, with the greatest risk conferred by exposure before the age of twenty [24]. In most irradiated populations, which include atomic bomb survivors, girls treated for scoliosis or tuberculosis, and women treated for mastitis, the type of breast cancer was not determined. A recent study from Milan of the molecular and marker analysis of the breast cancers of women exposed to therapeutic radiation for childhood/young adult cancers revealed a high risk of breast cancer diagnosed at an early age (39 compared to 57 in a sporadic consecutive series) and a higher frequency of ER-negative tumors [25]. Moreover half (53%) of the breast carcinomas from irradiated women showed features of basal-like tumors compared to 11% in a consecutive series of breast cancers not preceded by radiation. When compared to age-matched controls, basal-like cancer was significantly more frequent in irradiated women. The breast cancer subtype in young women treated with radiation was much more likely to be ER- and PR-negative and p53 and cytokeratin 5/6 positive and less likely to be HER2+ compared to girls who received radiotherapy [25]. Interestingly a disproportionate frequency of contralateral ER-negative breast cancer has not been noted in older women treated with radiation for breast cancer, suggesting a physiological basis for the shift identified in the Milan study.

Approximately 90% of the breast cancers in women who received irradiation for Hodgkin's lymphoma can be attributed to their radiation treatment [26]. This population provides a unique opportunity to determine whether radiation alters the course, as well as the frequency, of cancer. Broeks and colleagues used gene expression profiling to assess radiation-associated breast tumors (n=22) compared with a set of control breast tumors (n=20) of women unexposed to radiation, diagnosed at the same age [27]. Unsupervised hierarchical clustering of the profile data resulted in a clustering of the radiation-associated tumors separate from the control tumors. Consistent with the Milan study, tumors from irradiated women were often of the intrinsic basal breast tumor subtype. They also found a chromosomal instability profile and a higher expression of the proliferation marker Ki-67 suggesting a more aggressive tumor type. Since radiation induced DNA damage is random, the biological basis for the prevalence of this tumor type in irradiated women cannot be readily explained on the basis of the mutagenesis per se.

Radiation as a Carcinogen in Experimental Studies

Radiation is considered to be a complete carcinogen. DNA damage produces cells with oncogenic mutations and multicellular responses to DNA damage create a permissive environment for progression [12,28]. Either microenvironments that promote the malignant phenotype are in a way functionally equivalent to the acquisition of additional mutations in the initiated cell or, the irradiated microenvironment creates novel selective pressures

accelerate tumor development, or both. While DNA damage, the DNA damage response, and mutagenic consequences have been intensively characterized and are thought to be the root of radiation's action as a carcinogen, there are provocative studies that microenvironments induced by the response to radiation can promote neoplastic progression in unirradiated epithelial cells. These events "outside of the box" may significantly increase cancer risk.

A study by Kaplan and colleagues dating back more than 50 years demonstrates that radiation carcinogenesis is complex. These studies used C57BL mice, which are very susceptible to thymic lymphomas after radiation exposure. Young mice underwent thymectomy, and 2–7 d later received the first of four consecutive doses of 168 cGy. Several hours after the last irradiation, a single thymus from a non-irradiated mouse was transplanted subcutaneously under the right chest or upper abdomen of each of the previously thymectomized, irradiated hosts. Surprisingly, thymic lymphoma incidence and latency arising from the grafts matched that observed in irradiated, intact mice. Furthermore, the tumors were histologically identical to those found in the intact mice, and exhibited a similar pattern of metastasis [29]. This study showed that radiation-induced thymic lymphomas can occur even when the grafted thymus was never exposed to radiation, suggesting a systemic effect of radiation in the host.

This systemic mechanism of tumor induction was elucidated in their second study, which showed that shielding a thigh of the host during irradiation or promptly injecting fresh bone marrow into the host shortly after the last irradiation could neutralize the tumor-inducing effect of radiation [30]. The next study showed that prior radiation exposure impaired regeneration, which was mediated by bone marrow derived cells as thigh-shielded mice exhibited an identical degree of graft regeneration as observed in unirradiated mice [31]. The final publication provided conclusive evidence that the tumors that arose in the unirradiated thymic grafts were indeed composed of donor cells and not invading host cells that had received radiation by using FI hybrid mice [29]. The susceptible C57BL strain of mice was crossed with the C3H strain, which is resistant to radiation-induced lymphomas, to generate an F1 hybrid. These studies showed that the genetic background of the graft donor determined tumor incidence and thus, susceptibility was a property of the thymus, even though the mechanism of induction occurred through the host. This series of papers highlight the host as an effective target of radiation in the induction of thymic lymphomas in grafts that were never irradiated.

In a similar series of studies, Billingham and colleagues used the carcinogen methylcholanthrene to determine which compartment was the site of carcinogenic action in mouse skin. Skin grafts of various thicknesses (including or excluding hair follicles) from carcinogen-treated sites were transplanted to untreated sites in the same animal. Such an approach revealed that the underlying dermis layer conferred equivalent tumorigenic potential, even if the overlying epidermis was untreated. Tumors occurred when untreated grafts were transplanted into treated dermis, but not when treated grafts were placed into untreated dermis [32].

Morgan and colleagues showed that an immortal myogenic cell line formed tumors far more rapidly in irradiated compared to non-irradiated host muscle. The accelerated tumor phenotype was a direct effect of irradiation on the stroma, rather than due to systemic effects, because tumors did not form in distant muscle sites [33]. Interestingly, when transplanted to normal mice, these tumors formed large amounts of muscle. Likewise, irradiated pancreatic fibroblasts mixed with pancreatic carcinoma cells formed more aggressive and invasive cancer than when the pancreatic cancer cells were mixed with nonirradiated pancreatic fibroblasts [34]. These authors further demonstrated that an

antagonist of hepatocyte growth factor completely blocked the increased invasiveness of pancreatic cancer cells that was induced by co-culture with irradiated fibroblasts.

Even cell transformation, often ascribed to misrepaired DNA damage, is susceptible to microenvironment. The frequency of neoplastic transformation in cultured irradiated tracheal epithelial cells [35,36] or C3H 10 T1/2 cells [37] is inversely correlated to the number of cells seeded, i.e. the fewer cells seeded the more transformed colonies were evident, suggesting that cell density/interactions suppressed this supposedly mutagenic consequence. Bauer and colleagues also showed that the frequency of radiation, chemical and virally mediated transformation of cultured human and rodent fibroblasts is actively suppressed by non-transformed cells (reviewed in [38]). In a process called intercellular induction of apoptosis, non-transformed cells induce selective ablation of transformed cells via apoptosis [39]. If this control system acts in vivo as efficiently as it does in vitro, tumor formation should require the establishment of resistance mechanisms directed against intercellular induction of apoptosis. Indeed, transformed foci from cells cultured from established tumors are not influenced by the presence of normal cells [39].

Terzaghi-Howe showed that transforming growth factor β (TGFβ) produced by the differentiated normal epithelial cells inhibited the growth and phenotype of radiation-transformed cells [40]. Bauer described three distinct, but competing, roles for TGFβ during transformation (reviewed in [41]: TGFβ actually helps maintain the transformed state of mesenchymal cells, enables non-transformed neighbors to recognize transformed cells, and triggers an apoptosis-inducing signal. Bauer and colleagues showed that the latter two processes are enhanced following very low radiation doses [42].

The Role of Stroma in Radiogenic Mammary Cancer

The mammary glands of mice irradiated with 0.5–5 Gy show rapid changes in the stromal matrix [43–45]. Collagen type III is induced in the adipose stroma and peri-epithelial stroma, while collagen type I undergoes remodeling in the peri-epithelial stroma. Consistent with the immunoreactivity, production of new collagen fibrils is increased in the irradiated mouse mammary gland. Surprisingly, tenascin, which is down-regulated in adult mammary gland, is rapidly induced in the peri-epithelial stroma by radiation. Consistent with this wound-like stroma, TGFβ is activated and mediates much of the stromal remodeling [46]. In some ways, the irradiated microenvironment exhibits features of an ‘activated’ stroma, capable of further evolution that could modify the behavior and function of resident epithelial cells.

This rapid remodeling of the mammary microenvironment led us to hypothesize that the irradiated stroma augments breast cancer potential [2,43,47]. To test this, we created a radiation chimera by transplanting unirradiated, preneoplastic mammary cells to the mammary glands of irradiated hosts [10]. The mammary gland is uniquely suited to studies of stromal-epithelial interactions because the epithelium develops postnatally from a rudiment that is readily removed from the inguinal glands of female mice at 3 weeks of age. The so-called clearing of the fat pad results in a stable gland-free fat pad. Transplantation of normal mammary epithelial cells at the time of clearing produces a normal ductal outgrowth that fills the fat pad in 10 weeks and is indistinguishable in from intact gland by whole mounts or histological analysis (92).

In the radiation-chimera model, the mammary epithelium is surgically removed at puberty, the adult animal is irradiated some time later, and then non-irradiated mammary epithelial cells are transplanted into the irradiated host [10]. Initial studies used COMMA-1D mammary epithelial cells, which undergo mammary morphogenesis when transplanted into a 3-wk old mammary gland. They are non-tumorigenic if injected into the cleared fat pads of

3-wk old mice, subcutaneously in immature and adult mice, or into nude mice. Although clonal in origin, COMMA-1D cells harbor two mutant Trp53 alleles that may confer neoplastic potential [48]. When transplanted into mice irradiated 1–14 d earlier with 4 Gy, outgrowths rapidly developed tumors, ranging from a peak of 100% at day 3 and twice that of sham-irradiated mice at 14 d post-irradiation. Furthermore, tumors from irradiated animals were nearly five times larger than the few tumors that arose in sham-irradiated hosts, indicating that tumor biology, as well as frequency, was affected. These data support the idea that high dose radiation promotes carcinogenesis by inducing a hospitable tissue environment. The effect of the irradiated microenvironment on neoplastic progression persisted for several weeks and appears to be independent of systemic radiation effects (as tested by hemi-body irradiation), which support the hypothesis that non-mutagenic effects of radiation can contribute significantly to radiation carcinogenesis in vivo.

Similar observations have also been made for chemical carcinogenesis. Soto and colleagues showed that the stroma is a target of the n-methylnitrosurea (NMU) in the rat mammary gland [8]. NMU treatment of the rat mammary stroma promoted tumorigenesis of mammary epithelial cells that were not treated with the chemical carcinogen. In contrast, Medina and colleagues performed a similar experiment using 7,12-dimethylbenzanthrcene (DMBA)-treated mice and found that the treated mouse mammary stroma did not alter tumorigenesis by untreated preneoplastic mouse mammary outgrowth lines [49].

In preliminary studies (Nguyen et al., submitted), we used the radiation chimera transplanted with *Trp53 null* mammary epithelium to assess radiation dose dependence. The *Trp53 null* mammary epithelium progress from ductal outgrowths to ductal carcinoma in situ to invasive breast carcinomas over the course of a year and exhibit many features similar to those of human tumors, including genomic instability and heterogeneous tumor histology that can be either estrogen receptor (ER) positive or negative [50,51]. The experiments demonstrate that low dose (<1 Gy) host irradiation reduces tumor latency, but also affects the tumor type in a manner similar as to that found in breast tumors of women treated with radiation [25,27].

Mammary Composition and Radiation

Girls exposed to ionizing radiation at Nagasaki-Hiroshima who were peri-pubertal (approximately aged 10–14 years) were much more likely to develop breast cancer than older girls or adult women who were exposed to comparable radiation doses [52]. Similar results were found for high dose radiation exposures to the breast from fluoroscopy for radiation therapy for Hodgkin's disease [53–55]. A major question that arises from these epidemiological data is: What is the biological basis for the window of susceptibility during adolescence? Ongoing studies in our laboratory suggest that radiation exposure can affect stem cell self-renewal and that it may be mediated through the microenvironment.

It is becoming clearer that "stemness" as measured ex vivo is not a single property, but several properties that can be manifest under different conditions [56]. A stem cell is the mechanism for regenerating tissue after injury [57]. Hence, it must not only be capable of producing many differentiated progeny, but able to switch between these options when appropriate. Thus, the properties, and probably the number, of stem cells can change in response to circumstances, including experimental manipulations. A variety of recent studies in tissues ranging from brain to liver have brought to light the ability of partially-differentiated cells to dedifferentiate and replenish the supply of true stem cells. This degree of plasticity is unexpected based on the traditional understanding of stem-cell function [56], but may be relevant to the concern that these cells are particularly susceptible to changes in the stroma.

Recent studies in mouse mammary gland have provided new insight into how signaling between cells determines the activity of the stem cell pool and cell-fate decisions that determine mammary lineages. Studies from Visvader show that macrophages influence mammary stem cell activity and support stem/progenitor cell functions [58]. Some research suggests that bone marrow derived cells might contribute to the niche, at least in cancer [59]. It is clear that stromal signals are important; the best studied is Wnt/β-catenin. Wnt proteins are secreted morphogens that are required for cell-fate specification, progenitor-cell proliferation and the control of asymmetric cell division. Wnt binds to Frizzled receptors to induce β-catenin stabilization and translocation to the nucleus where it interacts with transcriptional factor TCF/Lef-1 [60]. MMTV-Wnt1 expands cell populations expressing CK4, Sca1, CK6, $CD24^{med}/49f^{high}$, and other profiles that have been associated with stem and early progenitor populations. Increased β-catenin transcriptional activity reduced latency in mammary tumor models, with tumors displaying a higher proportion of progenitor cell markers [61,62]. This experimental model supports the concept that increased stem cell number may itself be a major risk factor for cancer development [63].

A remarkable series of experiments by Smith and colleagues demonstrate that mammary epithelial cells can reprogram diverse cell types [64,65]. Transplantation into the mammary fat pad of normal mammary epithelial cells mixed with marked cells isolated from mature testis induce conversion or re-programming of the beta-galactosidase marked testis cells with mammary repopulating capacity. The idea that the niche can supersede established phenotype demonstrates that this population has a high degree of phenotypic plasticity [66]. We showed that a single radiation exposure predisposes human mammary epithelial cells to undergo TGFβ-mediated epithelial-to-mesenchymal transition (EMT) [67]. Recent studies from Weinberg indicate that transition through an EMT can endow otherwise differentiated cells with a stem-like phenotype [68]. Our in vitro model suggests that irradiation alters the response to TGFβ, which when taken together with our prior observations that radiation induces TGFβ activation, suggest that radiation can operate at multiple levels to deregulate stem cell pool size.

Concluding Remarks

Stroma is a critical player in carcinogenesis; indeed the normal stroma is a significant barrier to malignancy. An important question arises: how does stroma convert from a defensive to offensive player in tumorigenesis? Disruption of stromal-epithelial interactions is an understudied activity of carcinogens and a novel avenue through which to explore new strategies for intervening in the neoplastic process. Our studies using radiation show that that the mammary stroma can be activated by even a single radiation exposure, putting the stroma into play much earlier than generally thought in the carcinogenic process.

Since radiation is a well-documented human breast carcinogen, understanding its actions is essential for predicting risk medical exposures. A deeper understanding of radiation's action as a carcinogen might also reveal previously unsuspected routes to spontaneous cancer, just as the knowledge of its effect on DNA damage and responses has focused attention on DNA damage pathway gene polymorphisms in general populations. Moreover, the basic biology of radiation overlaps that of oxidative stress and can be used to probe tissue response to reactive oxygen species generated during other tissue processes like inflammation. Finally, understanding how radiation causes cancer may provide the means to protect children from the risk associated with exposures to necessary diagnostic procedures like computerized tomography scans, which can result in doses comparable to those in which risk has been measured, or from radiation treatment for other cancers.

Abbreviations

TGFβ	Transforming growth factor β
ER	estrogen receptor
PR	progesterone receptor
EMT	epithelial-mesenchymal transition

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