

Commentary

Nontransgenic models of breast cancer

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Abstract

Numerous models have been developed to address key elements in the biology of breast cancer development and progression. No model is ideal, but the most useful are those that reflect the natural history and histopathology of human disease, and allow for basic investigations into underlying cellular and molecular mechanisms. We describe two types of models: those that are directed toward early events in breast cancer development (hyperplastic alveolar nodules [HAN] murine model, MCF10AT human xenograft model); and those that seek to reflect the spectrum of metastatic disease (murine sister cell lines 67, 168, 4T07, 4T1). Collectively, these models provide cell lines that represent all of the sequential stages of progression in breast disease, which can be modified to test the effect of genetic changes.

Keywords: metastasis, progression, proliferative breast diseases, xenografts

Introduction

The development of animal models for breast cancer research covers a nearly 100-year span, starting with the pioneering work of Little, and later that of Strong, Heston, and Bittner, among others [1]. By selective inbreeding, numerous mouse strains were produced, with characteristic incidence frequencies and biologic characteristics of 'spontaneous' mammary tumors. Later, rat breast tumor models, frequently involving exposure to chemical carcinogens, were added to the roster of available animal tools. These rodent models were useful for testing hypotheses regarding the etiology of breast cancer (genetic, viral, environmental), for learning about factors that can play a role in breast cancer progression (hormonal, immunological), and for development of prevention and treatment strategies. The arrival of the immunodeficient nude mouse during the 1960s opened the door to the development of xenograft models of human breast cancer. More recently

the transgenic and knockout technologies have yielded mouse strains with specific genetic alternations or deficiencies that result in breast cancer development.

All of these models have strengths and weaknesses. Given the extensive population, genetic, and phenotypic heterogeneity of human breast cancers, none of the models can represent more than a very small fraction of the possible avenues by which human breast cancers develop, progress, or behave. The inbred mouse models are 'black boxes', in which the relevant mechanisms that underlie cancer development are highly complex and largely unknown. The chemical carcinogen or virus-associated models do not seem to mimic, at least superficially, the etiology of human cancer. The transgenic models, although mechanistically clearer than the others, often sacrifice complexity and the tempo of natural history. Furthermore, although they may show how gene alterations

can effect cancer development, they do not necessarily reflect how they do so under the 'normal' conditions of maturation, endocrinologic change and environmental exposures that occur during the long preclinical period during which a woman passes from being at risk to developing breast cancer.

In our minds, the 'ideal' *in vivo* model would meet several criteria. First, it would recapitulate the histopathologic spectrum and heterogeneity of breast neoplasia in women. It would also reproduce the natural history of the disease in terms of a prolonged and sporadic time span for development, with the variability in course that is characteristic of human cancer. In addition, it would lend itself to deciphering and confirming the cellular and molecular events, and the interactions among them, that are of relevance to human disease. Finally, it would achieve all of these objectives at an acceptable level of experimental convenience and cost. With some trepidation, we describe two types of model that, although certainly not ideal, have been designed to address two distinct areas of breast cancer biology: progression of proliferative breast disease to frank neoplasia, and metastasis of primary cancer to distant sites.

Preneoplastic models of breast cancer development

Most studies of preneoplasia and early progression of mammary tumors have utilized mouse HAN [2]. Medina [3] described a number of HAN lines that progress to carcinoma at different rates. When HAN tissue is transplanted into epithelium-free mammary fat pads, the transplanted epithelium expands to fill the mammary gland and resembles the normal mammary gland epithelium of pregnant mice. These HAN lines are not cultured cell lines, but rather tissue must be transplanted serially. The preneoplastic stage may be maintained indefinitely by serial transplantation but, if allowed to persist *in situ*, foci of carcinoma arise and rapidly growing tumors develop.

Although the HAN models are the basis for much information regarding the basic biology of mammary cancer, a number of differences in the histology and biology of mouse and human lesions exist. Unlike the HAN models, in which homogeneous lobuloalveolar lesions consistently give rise to rapidly growing adenocarcinomas within a few months, the breasts of women who are at high risk for proliferative breast disease are heterogeneous, and early breast cancer grows slowly.

The MCF10AT system is a xenograft model of progressive human proliferative breast disease. In this model the progression of a T24-*Ha-ras*-transformed derivative of normal-appearing MCF10A cells [4] (ie MCF10AneoT [5]) can be followed from a histologically precancerous stage to development of frank invasive carcinoma [6]. In contrast to MCF10A cells, MCF10AneoT cells form persistent lesions

in immunodeficient mice when 1×10^7 cells suspended in Matrigel are inoculated subcutaneously [6]. MCF10AneoT cells and lines derived by alternating *in vivo* transplantation and *in vitro* culture (MCF10ATn) are collectively known as the MCF10AT system [7]. MCF10AT cells grow in immunodeficient mice, in which, over a period of several months, a percentage of lesions undergo a sequence of progressive histologic changes. These changes mimic those observed in the breasts of women who are at high risk for breast cancer, and culminate in a significant proportion of grafts with frankly invasive carcinoma. The lesions formed by lines of the MCF10AT system are composed of a heterogeneous spectrum of ductular tissues with a range of morphology that includes mild hyperplasia, moderate hyperplasia, atypical ductal hyperplasia (ADH), carcinoma *in situ*, moderately differentiated and undifferentiated carcinoma, and histologically normal ducts.

Although it may be argued that the presence of mutant *Ha-ras* gene, a rare mutational event in human breast cancers, may have contributed to the transformation process by initiation and/or selection of a subpopulation of MCF10A cells, the presence of mutant *Ha-ras* is clearly not sufficient for histologic progression of MCF10AT cells. Indeed, MCF10AneoT clones that express high levels of protein encoded by mutant *Ha-ras* have been shown to lack the ability to form lesions [8]. Furthermore, 50% of human breast carcinomas express elevated levels of the protein encoded by normal *Ha-ras* [9,10].

An important feature that distinguishes MCF10AT cells from parental MCF10A cells is the presence of a functional wild-type estrogen receptor (ER). MCF10AT cells are able to respond to estrogen treatment *in vitro* with increase in size and ability to form colonies on soft agar [11,12] and *in vivo* with rapid morphological conversion to ADH and ductal carcinoma *in situ* (DCIS) [13]. The effects of estrogen on histologic progression to ADH and DCIS appear to be ER-mediated, because treatment of animals with tamoxifen causes specific suppression of progression to ADH and DCIS [14]. Also, the highest levels of ER in human breast tumors are generally observed in atypia and nonhigh-grade DCIS [15].

Much like human breast cancers that have lengthy natural histories, the lesions produced by premalignant MCF10AT xenografts are slow growing and not yet committed to a single pathway of cancer unless they are manipulated by hormonal supplementation. Remarkable features of the MCF10AT system are the reproducible generation of premalignant lesions and the few cytogenetic alterations present in the various MCF10AT generations that are not already present in the parental MCF10A cells [4,6,16]. Establishment of tumorigenic variants of MCF10AT xenografts has been difficult. However, serial trocar passage of small pieces of MCF10AT lesions have yielded

tumorigenic variants that produce heterogeneous tumors with prominent areas of DCIS and invasive carcinoma. One of the 14 clones derived from this variant reproducibly generates tumors with predominant comedo DCIS (MCF10DCIS.com) within a few weeks [17,18].

Breast cancer is a heterogeneous disease, and the heterogeneous spectrum of disease progression exhibited by this model is indicative of its multipotentiality. The absence of commitment to a single pathway of cancer, and its easy manipulability by hormonal agents, render the MCF10AT xenograft model the only currently available human model that has been shown to exhibit the histologic stigmata identified in women who are at high risk for developing breast cancer, and furthermore to undergo preneoplastic and neoplastic progression *in vivo*.

The invasive carcinomas generated by MCF10AT xenografts are themselves heterogeneous. Different histologic differentiation (squamous, glandular, and undifferentiated) is seen, as well as distinctive immunohistochemical staining for breast cancer-associated markers such as erbB2, ER, cyclin D₁, mucins, keratins, and p53. Therefore, although the cancers that develop from this single cell line derived from a single patient may represent only a subset of human breast cancer, the model is not limited to a single cancer phenotype.

Metastatic breast cancer models

Although there are a number of human breast cancer lines that will metastasize in the xenograft setting, none fully reflect the spectrum of metastatic disease in humans. Numerous laboratories have been able to obtain subpopulations from both rat and mouse mammary tumors that differ in metastatic capacity [19–21]. Generally, these models consist of paired subpopulations, one of which is highly metastatic and the other not, but specific deficiencies of the nonmetastatic variants are usually unknown, and so the mechanisms of metastatic failure are obscure. The metastatic process is a sequence of steps (invasion, intravasation, transport, arrest, extravasation, and growth) that must be accomplished by cancer cells before distant metastases are established. Nonmetastatic cell lines are unable to complete one or more steps in the metastatic cascade, whereas metastatic cell lines must be able to complete all of them. In order to follow the sequential spread and replication of tumor cells, a sensitive method to determine the presence of clonogenic tumor cells in host tissue is required. This can be accomplished by using tumor cells that are resistant to specific drugs and clonogenic assays in drug-containing selective medium that quickly kills host cells but not tumor cells.

Tumor subpopulation lines 67, 168, 66, and 410.4 were isolated from a single, spontaneously arising mammary tumor from a Balb/c/c3H mouse. Sublines 168FAR

(diaminopurine-resistant) and 66C14 (thioguanine-resistant) were selected from the parental populations 168 and 66, respectively, and sublines 4T1 and 4T07 (both thioguanine-resistant) were derived from the parental population 410.4. The geneticin-resistant subline 67NR was obtained by transfection of line 67. These subpopulations are phenotypically heterogeneous for a number of characteristics but share a common origin. Subpopulations are classified as metastatic on the basis of their ability to metastasize spontaneously from the orthotopic site [22,23]. One line, 4T1, metastasizes to the lung, liver, bone, and brain via the hematogenous route, whereas 66c14 metastasizes to the lung and liver via the lymphatics. Sublines 67, 168FAR and 4T07 are highly tumorigenic, but fail to metastasize at different steps. The nonmetastatic 67NR cells fail to leave the primary site; 168FAR cells reach the regional lymph nodes but fail to produce nodules and do not advance past the nodes; and, although 4T07 cells may be recovered from the blood and lungs, visible metastases never develop. Thus, this comprehensive set of sublines offers the potential to correlate specific genetic alterations with specific steps in the metastatic process, as well as to test antimetastatic therapies for their ability to interfere with known stages of the process. The metastatic lines show a distribution similar to that of human breast cancer. In addition, 4T1 is one of the very few lines of any origin that spontaneously metastasizes to bone.

Conclusion

We describe two nontransgenic, *in vivo* models for studying breast cancer development. The models reflect two stages of the disease as they are seen in women. The preclinical MCF10AT model recapitulates the histologic spectrum of proliferative breast disease in women who are at high risk for developing invasive cancer. The metastatic model allows for investigation into mechanisms that are relevant to the routes and distribution patterns seen in women with systemic disease. We believe that models such as these, which are centered on the biology of the disease, can be used to guide research into the eventual eradication of breast cancer as a significant medical problem.

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